Immediate and Enduring Effects of Corticosterone Administration During Adolescence and Adulthood on Anxiety and Depressive Behavior in Male Rats

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Abstract

Previous research has shown that the stress hormone corticosterone can increase depressive and anxiety-like behavior in rats as well as dampen the HPA response to a novel stressor (Kalynchuk et al., 2004; Johnson et al., 2006). Several studies have also shown that adolescence is a period of increased sensitivity to the negative effects of stressors (reviewed in McCormick et al., 2010), which are often the result of exposure to corticosterone, and yet there is no research to date examining the effects of corticosterone administration during adolescence. The purpose of these experiments is to determine both the immediate and enduring effects of prolonged exposure to corticosterone in adolescence and adulthood on anxiety-like behavior, depressive behavior, and the HPA response. In Experiment 1 adolescent and adult rats were administered an injection of 40 mg/kg of corticosterone or vehicle daily for 16 days. Half of the rats were then tested on the elevated plus maze (EPM) one day after their last injection, and the following day were tested on the forced swim test (FST). After the FST, which is a stressor, blood samples were collected at three time points, and the plasma concentrations of corticosterone were determined using a radioimmunoassay. The remaining rats were left undisturbed for three weeks, and then underwent the same testing as the first group. Corticosterone treatment had little effect on anxiety-like and depressive behavior, but it did alter the HPA response to the FST. In those rats tested soon after the period of injections, corticosterone dampened the HPA response as compared to vehicle treated rats in both adolescent and adult treated rats. For the adolescent treated rats that were tested several weeks later, corticosterone treatment increased HPA response as compared to the vehicle treated rats, but the same was not true for the adult treated rats. It was hypothesized that the lack of behavioral

effects of the corticosterone treatment may be the result of the vehicle injections inducing a stress response and thereby both groups would have similarly altered behavior. In Experiment 2 rats were administered corticosterone dissolved in their drinking water with 2.5% ethanol, or just the 2.5% ethanol or plain water, to determine the effects of corticosterone treatment without a stressor present. The regular drinking water was replaced with treated water for 16 days either during adulthood or adolescence, and as before, rats were either tested in the FST one day after the water was removed or three weeks later. Again there was no effect of treatment on depressive behavior. Similar to what was observed in Experiment 1, corticosterone treatment dampened the HPA response to a stressor for the rats tested soon after the treatment period. However, in Experiment 2 there was no effect of treatment on HPA response in those rats tested several weeks after they were treated. These results indicate that corticosterone can have a lasting effect on the HPA when administered in adolescence by injections but not in drinking water, which is likely because of the different schedules of exposure and rates of absorption between the two administration methods.

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Introduction

The peak age for the onset of mental health disorders such as depression, anxiety disorders, schizophrenia and eating disorders like anorexia nervosa is 14 years old, during adolescence (reviewed in Giedd et al., 2008). Depression and anxiety disorders have been linked to stress and stress related hormones (reviewed in Parker et al., 2003). Adolescents have been shown to be more susceptible to the long term effects of stress than adults (reviewed in Lupien et al., 2009; McCormick et al., 2010). Increased susceptibility to stress during adolescence is likely responsible for the young age of onset for stress related mood disorders. However there is scant research available that addresses the question of why adolescents are more susceptible to the long term effects of stress, and there is no research to date that directly studies the effects of exposure to stress hormones during adolescence. The experiments described in this thesis will address the immediate and long term effects of administering corticosterone, the major stress hormone in that rat, during both adolescence and adulthood on anxiety-like behavior, depressive behavior, and the stress response in the rat. Animal models are necessary in the study of stress because they allow a level of experimental control that is not possible ethically in studies involving people. Experiments using animal models allow us to interpret correlation studies with people more accurately, and provide the base for informed hypotheses relating to human disorders. A general overview of the stress system, the hypothalamic-pituitary-adrenal axis, of the rat will be given. Animal models of anxiety and depressive behavior will be discussed as well as the effects of stress on these behaviors. This will be followed by a discussion of adolescence, and the ways in which adolescent rats differ from adult rats in terms of their stress response. How stress and corticosterone, one of the key hormones of the stress response, can affect anxiety and depressive behaviors in adolescents will then be discussed. The current experiment will

determine if exogenous corticosterone administered during adolescence will have a lasting effect on emotional behaviors and the stress response. Corticosterone should increase depression and anxiety-like behavior, and that corticosterone will alter the stress response. When the corticosterone is presented during adolescence, a time of ongoing brain development, these effects should be long lasting.

Hypothalamic-Pituitary-Adrenal Axis of the Rat

One of the functions of the hypothalamic-pituitary-adrenal (HPA) axis is to respond to aversive and potentially harmful situations to maintain homeostasis (reviewed in Herman & Cullinan, 1997). A stressor will activate the paraventricular nucleus of the hypothalamus where neurons will release corticotropin releasing hormone, which will travel to the pituitary gland. The pituitary will then release adrenocorticotropic hormone into the blood stream, which will travel to the adrenal gland. The adrenal cortex produces and releases corticosterone when it detects an adrenocorticotropic hormone. The amount of corticosterone present in the blood stream is common measure of the intensity of a stressor. For example, when exposed to electric shock, increased shock intensity induced a larger release of corticosterone resulting in higher blood plasma concentrations of corticosterone (Cordero et al., 1998). The HPA can be activated by several different stressors such as restraint, isolation, tail shock, swim, elevated platform, predator odor, direct predator exposure, social instability or a combination of several of these and possibly others referred to as chronic unpredictable stress. Each of these stressors triggers corticosterone release, which activates receptors in several brain areas for a variety of effects.

There are two types of receptors for corticosterone present in the rat brain: glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs). MRs have a ten fold higher affinity for corticosterone than GRs do. Therefore at low concentrations of corticosterone the majority of the

MRs (89.5%) at morning trough when plasma corticosterone concentrations were 1.4 μg per 100 mL, will be occupied but not many GRs (15%) (Reul & de Kloet, 1985; Spencer et al., 1990). At higher concentrations of corticosterone, such as those present after experiencing a stressor, GRs will become occupied in addition to MRs (Reul & de Kloet, 1985). For this reason MR activation is associated generally with basal levels of corticosterone and GR activation is associated generally with the stress response. GRs are found both in the cytosol and in the nuclear envelope, and when corticosterone is bound the receptor will relocate to the nucleus and bind to DNA (Spiga & Lightman, 2009). Activated GR will increase transcription in a dose dependent manner, and following a specific timeline (Spiga & Lightman, 2009; Sarabdjitsingh et al., 2009). Both MRs and GRs are found in high concentrations in the hypothalamus, hippocampus, amygdala, and medial prefrontal cortex (reviewed in de Kloet et al., 1998).

When high levels of corticosterone activate GRs, the hypothalamus will be inhibited resulting in reduced release of adrenocorticotropic hormone, which in turn prevents further release and production of corticosterone (reviewed in de Kloet et al., 1998). This process is known as negative feedback, and it limits the duration and magnitude of corticosterone release (reviewed in Herrman et al., 2009). One important site of negative feedback is the hippocampus, and when there is a large quantity of GRs in the hippocampus the HPA is tightly controlled, and the reverse is true as well (reviewed in de Kloet et al., 1998). Repeated exposure to corticosterone can damage the hippocampus (Woolley et al., 1990; Watanabe et al., 1992; Galea et al., 1997; Magarinos et al., 1998), which would likely reduce its control of the HPA.

In addition to negative feedback, another mechanism for preventing excessive exposure to corticosterone is habituation. Habituation refers to a rats' ability to attenuate the release of corticosterone to a stressor that is presented repeatedly. This effect is stressor specific in that a rat

that has habituated to one stressor, for example intermittent cold presented for seven days, would show decreased corticosterone release in response to cold but not in response to a novel stressor, such as restraint (Bhatnagar & Dallman, 1998). Habituation depends on the predictability of the stressor, and therefore it is context specific (Grissom et al., 2007). Blocking both MRs and GRs, or MRs alone, can impair habituation, which indicates that receptors play an important role in habituation (Cole et al., 2000). Although stressors will activate the HPA causing corticosterone release there are several mechanisms in place to prevent extensive exposure to corticosterone because corticosterone can disrupt emotional processing giving rise to changes in anxiety and depression-like behaviors.

Stress, Corticosterone, and Anxiety

Anxiety and fear share several causes, behaviors, and related brain structures, but anxiety can be distinguished from fear in several ways. Anxiety is a prolonged state of apprehension, whereas fear is a brief reaction that dissipates with the removal of the fearful stimuli. Anxiety is often elicited by a distant or potential threat, but fear is cued by an immediate clear threat (reviewed in Davis et al., 2010b). When a fearful stimulus is present the basolateral nucleus of the amygdala signals the central nucleus of the amygdala, which in turn signals the hypothalamus and the brainstem to produce an initial fear response. When that stimulus remains present for a prolonged period of time, the central nucleus of the amygdala releases corticotropin releasing hormone to the bed nucleus of the stria terminalis, which creates an anxious reaction. At the same time inhibitory feedback within the amygdaloid nuclei terminates the fear response allowing for the transition from specific fear to generalized anxiety (reviewed in Davis et al., 2010b). Increased dendritic arborization in the amygdala has been linked to increased anxiety (Mitra & Sapolsky, 2008). Anxiety behaviors are both produced and mediated by the amygdaloid

nuclei, and therefore altered levels of anxiety may be indicative of disrupted processing or damage to the amygdaloid nuclei.

A very common and well validated measure of anxiety in rodents is the elevated plus maze (EPM), first described by Pellow et al. (1985) (Wall & Messier, 2001), and the review of stressor and corticosterone effects on anxiety will be limited to studies that have used this measure. The EPM relies on the propensity of rodents to explore novel territory and their fear of open and elevated areas. The EPM consists of four arms elevated off the ground. Two arms are closed, with tall walls surrounding the edges, and two are open with no walls on the edges. The time spent in an open arm indicates the level of anxiety; more time would signify a less anxious animal. Rats will inherently spend more time in the closed arms than the open arms, but the amount of time spent in the open arm can be increased or decreased with anxiolytic and anxiogenic drugs respectively (Pellow et al., 1985). Rats show increased plasma corticosterone concentrations when confined to the open arm, which is evidence that the open arm is aversive or stressful (Pellow et al., 1985; Avital & Richter-Levin, 2005; Tsoory & Richter-Levin, 2005; Tsoory et al 2007; Toth et al 2008; McCormick et al 2008; Zamudio et al 2009).

One study using factor analysis described three main behavioral factors measured by the EPM, anxiety, risk assessment (which is also referred to as decision making) and activity (Doremus et al., 2006). However, comparisons across factor analysis studies and other studies show that the factor of activity is ambiguous (rev in Wall & Messier, 2001). The behavioral measure that best describes activity in the EPM is not agreed upon, and whereas some use total arm entries as the measure of activity and others use closed entries, both of these variables load on the factor of anxiety in addition to the factor of activity (rev in Wall & Messier, 2001). There is no measure of activity on the EPM that is not influenced by anxiety to some degree. However,

closed entries are less influenced by anxiety than total entries because the measure of total entries includes the entries into the open arm, which is a direct measure of anxiety. Whereas some factor analyses have shown that a factor of risk assessment should be included in the measures of behavior in the EPM (e.g. Doremus et al., 2006), others argue that the variables that measure risk assessment are merely identifying elements of other constructs (Wall & Messier, 2001). Although anxiety is the factor that all analyses agree upon, activity and risk assessment are less concurrent factors for the EPM.

The majority of the literature shows that stress, both chronic and acute, increases anxiety as measured by the EPM. Studies have reported increased anxiety in the EPM in rats 24 hours after, four hours after, or immediately after one session of restraint (Calvo et al., 2001; Lunga & Herbert, 2004; Gameiro et al., 2006). When tested immediately following exposure to a compartment that had previously been paired with shock, rats showed increased anxiety on the EPM compared to control rats not exposed to the compartment (Gameiro et al., 2006). Rats exposed to a predator odor (used cat litter), showed increased anxiety on the EPM (Tsoory et al., 2007). Acute stress can increase anxiety in the EPM across a range of time intervals between the stressor and the test.

Several studies have examined the effects of chronic exposures to stress on anxiety, and predominantly report the same general effect of chronic stress on anxiety as for acute stress. Chronic restraint of various durations, six hours a day for one week (Bowman et al., 2009), one hour daily for three or five days (Gameiro et al., 2006), or two hours a day for 10 days (Vyas et al., 2004) enhanced anxiety on the EPM. Restraint for one hour a day, five days a week, for 50 days also increased anxiety (Noschang et al., 2009). Chronic unpredictable stress, a paradigm that involves changing the type of stressor from day to day to prevent habituation, increased

anxiety when the procedure continued for either two or four weeks (Bondi et al., 2008; Pego et al., 2008). These studies show that stress can increase anxiety as measured by the EPM.

In contrast to the above studies finding a relationship between stress and heightened anxiety, some studies have reported various stressors to decrease anxiety or have no effect on anxiety in the EPM. Mice tested the day after exposed once to a cat showed decreased anxiety on the EPM compared to controls in one study (Adamec et al., 2006), and no change in anxiety but increased risk assessment compared to controls in another study (Adamec et al., 2004). However, other studies have found that predator odor may or may not produce an effect on behavior depending on the particular cat used as a stimulus (Munoz-Abellan et al., 2009; Munoz-Abellan et al., 2010). Therefore it is impossible to determine whether the behavior was unaffected by the stressor or if the particular cat used in this study was an ineffective stressor. Chronic unpredictable stress for a duration of one week produced a decrease in anxiety compared to unstressed controls (Kompagne et al., 2008) and another study found no effect of chronic unpredictable stress presented for either one, seven or 14 days, on behavior in the EPM when compared to untreated controls (Matusewich et al., 2007). These discrepancies might be caused by the duration of the stressor, because as noted above, unpredictable stress increased anxiety when it lasted for two or four weeks (Bondi et al., 2008; Pego et al., 2008). Furthermore, unpredictable stress models are difficult to compare to other studies because the stressors are not constant, and in fact the schedule of stressors and variety of stressors used changes from study to study. The wide variety in these procedures makes them difficult to compare one to another or with studies using the same stressor repeatedly. Thus, although there are examples of stress exposures decreasing anxiety, they are much less prevalent than studies that show stressors to increase anxiety.

Exogenous administrations of corticosterone generally mimic the effects of stressors on anxiety. Daily injections of 25 mg/kg of corticosterone to rats for 28 days increased anxiety on the EPM (Pego et al., 2008), whereas administration of a GR antagonist to rats decreased anxiety on the EPM (Korte et al., 1995), which confirms the anxiogenic properties of corticosterone. Removing the adrenal gland, the main source of endogenous corticosterone, abolishes the increase in anxiety caused by stress, and corticosterone replacement or treatment with GR or MR agonists will reinstate it (Calvo et al., 2001). Treatment with metyrapone, a drug that prevents the synthesis of corticosterone, can also extinguish the increase in anxiety caused by stress, and treatment with the GR agonist, dexamethasone, will reinstate it (Calvo et al., 2001). These studies indicate that the stress-induced increase in anxiety can be attributed to the release of corticosterone associated with stress.

Several studies suggest that the amygdala is the locus of action for corticosterone's anxiogenic effects. One systemic injection of 10 mg/kg corticosterone increased anxiety in rats compared to vehicle injected controls, and the increased anxiety coincided with increased arborization in the basolateral amygdala (Mitra & Sapolsky, 2008). Exposure to stressors also increases spine density and enhances dendritic arborization in the basolateral nucleus of the amygdala (Mitra et al., 2005; Vyas et al., 2002). Anxiety in the EPM is increased when corticosterone is directly applied to the central nucleus of the amygdala (Shepard et al., 2000). These studies indicate that the amygdala is the region responsible for the effect of stress and corticosterone on anxiety.

The effect of stress on anxiety appears to be a lasting effect. Increased anxiety resulting from stress can still be observed after a 21 day recovery period, though to a lesser extent than that in rats tested immediately following the stressor (Vyas et al., 2004). When corticosterone (50

μg/mL) was given to rats in their drinking water for 14 days followed by six days of a weaning procedure whereby the dose was decreased incrementally, no effect was observed on EPM when tested either two weeks or one month after corticosterone treatment (Gourley & Taylor, 2009). One injection of 10 mg/kg of corticosterone, but not 10 daily injections, increased anxiety in the EPM when tested 12 days later (Mitra & Sapolsky, 2008). However, the chronic group was tested two days after the administration of corticosterone instead of 12 days later. It remains unclear whether the differences in results reported in these studies results from different durations of administration, or doses, or other differences between these procedures. More investigation into the long term effects of stress and corticosterone is necessary to clarify the point.

Forced Swim Test: A Measure of Depressive Behavior

Like increased anxiety levels, another indication of disrupted or altered emotional regulation is increased depressive behavior. Depression is a disorder with symptoms including feelings of sadness and helplessness, low self esteem, anhedonia, a lack of energy, insomnia, poor concentration, and an overall lack of positive emotions. Absence of happiness is a more consistent symptom of depression than increased sadness (Peeters et al., 2003). Additionally most patients with anxiety disorders have one or more depressive disorders, and patients with both disorders have increased severity of their principle disorder (Davis et al., 2010a). One of the first theories describing the underlying mechanism for depression is the monoamine theory, which states that depression is derived from to an imbalance in the neurotransmitter serotonin. One common class of antidepressants is selective serotonin re-uptake inhibitors, which prevent serotonin from being reabsorbed into the cell allowing it to remain in the synaptic cleft longer. A second common class of antidepressants is monoamine oxidase inhibitors, which inhibit the enzyme that decomposes serotonin, which also allows serotonin to remain in the synaptic cleft

longer. A newer theory points to the observation that depressed patients show evidence of a more active HPA axis such as increased plasma levels of cortisol, the human correlate of corticosterone (reviewed in Parker et al., 2003). Both of the aforementioned classes of antidepressants in addition to altering serotonin levels will also normalize a patients' cortisol levels (reviewed in Parker et al., 2003). The onset of depression is often preceded by an increase in stress or a particularly stressful event, and stress can exacerbate the symptoms of depression (reviewed in Parker et al., 2003). Depression is also linked with decreased hippocampal volume, decreased prefrontal cortex volume, and altered amygdala volume, in that the nuclei of the amygdala are larger soon after the onset of depression but decrease with the progression of the disorder (reviewed in Sterner & Kalynchuk, 2010). It is possible that these structural changes are results of a more active HPA, but because the hippocampus plays a role in HPA axis regulation it is also possible that having a smaller hippocampus to begin with causes a more active HPA axis. Further research is required to determine which of these characteristics of depression might contribute to the mechanism for the disorder.

Animal models can provide unique insight into the mechanisms behind disorders like depression. The forced swim test (FST) is a well validated measure of depressive behavior in the rodent. Although there are other tests of depressive behavior, this paper will only discuss the FST because of its reliability and widespread usage as a measure. Originally described by Porsolt and colleagues (1977), the test consists of a tall cylindrical tank filled with enough water such that the rat cannot stand on the bottom. The test consists of two sessions, a training session in which the animal is put in the tank for 15 to 20 minutes, and a second session 24 h later in which the rat is placed in the tank again for five minutes. The purpose of the first session is to induce depressive-like behavior by providing the animal with an inescapable stressor, water. During the

second session the amount of time the rat spent floating or moving just enough to keep its head above water, a behavior labeled immobility, was considered an index of depressive behavior. Spending more time immobile and becoming immobile sooner is indicative of a higher level of depression like behavior, and several studies have shown that a wide variety of antidepressants will decrease immobility when administered between the two sessions (Porsolt et al., 1977; Armario et al., 1988; Detke et al., 1995; Tejani-Butt et al., 2003; Pechnick et al., 2008). Other factors that have been shown to decrease immobility include electroconvulsive shock, REM sleep deprivation, and environmental enrichment (Porsolt et al., 1978). Later studies began to measure time spent actively swimming around the tank, time spent climbing the sides of the tank and time spent immobile separately.

Whereas every class of antidepressant has been shown to decrease immobility on the FST, swimming and climbing represent distinct forms of non-depressive behavior, and they are differentially affected by separate classes of antidepressants (Porsolt et al., 1977; Armario et al., 1988; Tejani-Butt et al., 2003; Pechnick et al., 2008; Detke et al., 1995). Serotonin re-uptake inhibitors will increase the amount of time spent swimming whereas drugs related to norepinephrine will increase the amount of climbing, and both subsequently decrease the amount of time spent immobile (Detke et al., 1995). Another study later replicated the norepinephrine-related drug-induced increase in climbing in both adult (postnatal day 112) and young (postnatal day 30) rats (Pechnick et al., 2008). A further study replicated the finding that serotonin related drugs increased swimming in young rats, postnatal day 21 (Reed et al., 2008). Another study reported that reducing serotonin signaling in mice through the use of antisense mRNA for the serotonin 2A receptor resulted in increased time spent immobile in the FST compared to control

mice (Sibille et al., 1997). These studies show that climbing and swimming behaviors in the FST are tied to different neurotransmitter systems.

Several types of chronic stressor procedures have been found to increase immobility in the FST. Restraint for two hours a day for one day, but not three or seven days, increased immobility during the first session of the FST, and seven days of restraint but not three or one days of restraint increased immobility on the second session of the FST (Cancela et al., 1991). Chronic unpredictable stress of various durations and using a variety of stressors increased immobility on the first session of the FST (Echandia et al., 1988; Molina et al., 1994; Kompagne et al., 2004). Several of these studies also show that antidepressants can ameliorate the effects of stress on behavior in the FST when they are given either after the stressor or directly before testing (Cancela et al., 1991; Molina et al., 1994). These studies demonstrate that stress can induce a depression-like state in rats as observed in the FST.

Studies of the effects of acute stressors on behavior in the FST have less consistent findings than do those of the effects of repeated stressors. Both acute restraint and periodic tail shocks for one hour immediately prior to testing decreased immobility in a single session of the FST, but no effect on immobility was observed after loud noise stress or when a stressor was administered 24 hours before the test session (Armario et al., 1991). In contrast, another study found no effect of two hours of periodic tail shocks on a single session of the FST, but in this study an anxiety test, the hole and board test, was performed between the shock and the FST, which may have affected the outcome of the FST (Pol et al., 1992). The same study found that two hours of restraint increased immobility, and previous chronic exposure to immobilization exacerbated this effect (Pol et al., 1992). Yet another study showed that resident intruder stress increased immobility in a single session of the FST when tested 24 hours later, but there was no

effect of six periodic foot shocks (Kavushansky et al., 2009). Potentially the reason for these discrepancies in the FST in relation to acute stressors is that the levels of circulating stress hormones have been dissociated from behavior on the task. Behavior during the FST has been shown to be the same for rats with different levels of circulating hormones (Walker et al., 1995; Johnson et al., 2006). Therefore the mechanisms underlying the behavioral effects of stress are more complicated than a direct response to hormone levels and less likely to be observed after an acute treatment. Additionally the length of time between the stress exposure and the test may have an effect on the outcome. More research is required to adequately explain the effects of acute stress on the FST.

Unlike acute stress, chronic stress consistently is found to increase depressive behavior in the FST (Echandia et al., 1988; Cancela et a., 1991; Molina et al., 1994; Kompagne et al., 2004; Maniam & Morris, 2010). Like other effects of chronic stress, this is likely the result of prolonged exposure to corticosterone. Chronic injections of 40 mg/kg of corticosterone for 21 days increased immobility in a single session of the FST for both males and females (Kalynchuk et al., 2004; Gregus et al., 2005; Johnson et al., 2006). Another study found that 19 days of 40 mg/kg of corticosterone via subcutaneous injection was sufficient to decrease immobility (Lee et al., 2009). The effect of corticosterone on immobility in the FST is dose dependent, and is observed using 40 mg/kg, but not 20 or 10 mg/kg of corticosterone (Johnson et al., 2006). Further, an acute dose of 10, 20, or 40 mg/kg had no effect on a single session of the FST when tested 24 hours after the injection (Johnson et al., 2006). The 21 days of 40 mg/kg corticosterone injections produced an increase in immobility but 21 days of six hours of restraint did not affect immobility (Gregus et al., 2005). The lack of a stress effect here may be caused by habituation to the stressor, which would result in lower levels of circulating corticosterone, because

corticosterone injections produced the expected effect. The effects of corticosterone in the FST may be long lasting. A study that provided mice with 50 μg/mL corticosterone in the drinking water for 14 days with a six day weaning procedure (whereby the dose was incrementally decreased) found increased immobility in the FST two weeks later (Gourley & Taylor, 2009). The duration of this effect may be the result of the unique schedule and dose, but it might be the case that the effect of corticosterone on depressive behavior is long lasting. Further research is required to determine if the effects of corticosterone on the FST are consistently long lasting.

Wistar-Kyoto rats have a higher basal level of corticosterone compared to Wistar or Sprague-Dawley rats, and Wistar-Kyoto rats spend more time immobile than Wistar or Sprague-Dawley rats in a single session of the FST (Tejani-Butt et al., 2003). A low zinc diet for two weeks increases corticosterone response to a stressor and also increased immobility in a single session of the FST (Watanabe et al., 2010). Because these studies show that enhanced corticosterone levels coincide with increased immobility on the FST, they also support the idea that the stress-induced increase in immobility on the FST is related to higher exposures to corticosterone.

There is much evidence that stress and high levels of corticosterone increase anxiety-like and depressive behavior. However, thus far all of the studies discussed only involved adults. Many anxiety and depression-related disorders are first manifested during adolescence, and therefore the role of stressors experienced during adolescence, a time of increased brain development, requires greater investigation. I will review the basics of the rat model of adolescence, and then I will discuss the research relating to the effects of stress on depressive and anxiety-like behavior in adolescence that is available thus far.

Adolescence

Adolescence is a period of ongoing development in the brain and of marked differences in behavior as compared to younger and older individuals (reviewed in Spear, 2000b). As for people, there is no precise onset or offset of adolescence in the rat. There are three stages considered to represent adolescence in the rat. There is a prepubescent early stage from postnatal day 21 through 34 (rats are usually weaned at day 21), a mid adolescent period from day 34 to 46, and a late adolescent stage from day 47 to 60 (reviewed in McCormick & Mathews, 2007). Rats older than day 60 are commonly agreed to be adults because adult-like sexual behavior has developed and rats are physically mature at this age (reviewed in McCormick & Mathews, 2007). Adolescence includes sexual maturation, but that is only one aspect of the development occurring in adolescence. The prefrontal cortex, hippocampus, and amygdala all undergo development during adolescence (reviewed in Lupien et al., 2009). The HPA axis also is continuing to develop in adolescence (reviewed in McCormick & Mathews, 2007). Adolescence is also a time of altered behavior, and behaviors such as social play and risk taking are increased during this time period compared to both older and younger rats (reviewed in Spear, 2000a).

HPA Activation During Adolescence

Adolescents differ from adults both behaviorally and in their physiological response to stressors, depending on the type and duration of the stressor. Several studies have shown adolescent rats to have more prolonged release of corticosterone in response to 30 minutes of restraint (Romeo et al., 2004a; Romeo et al., 2004b; Romeo et al., 2006; McCormick et al., 2008; Doremus et al., 2009b; Goldman et al., 1973; Vazquez & Akil, 1993; Novak et al., 2007). Although adolescents show an increased HPA response to a stressor, they will habituate with repeated presentations, like adults do. One study found that the release of adrenocorticotropic hormone was higher in adolescents only on the first of three days of restraint for three hours a

day, and the release of corticosterone was higher only on the second day (Gomez et al., 2002). After seven days of restraint for 30 minutes adolescents had a higher corticosterone and adrenocorticotropic hormone response to the same stressor, restraint, which would indicate less habituation than adults (Romeo et al., 2006). Not all stressors have the same effect during adolescence. A social stressor, changing cage partners, in combination with isolation induced a greater stress response at day 30 than isolation alone, and when presented for 16 days, the social stressor showed less habituation of the corticosterone response compared to isolation alone (McCormick et al., 2007). Repeated stressors presented during adolescence clearly have an effect on the HPA, but whether that effect is similar to adults depends on the type and schedule of the stressor.

Several studies have looked at the lasting effects of stress during adolescence on the HPA axis. The type of stressor, timing of presentation and time elapsed between the last stress presentation and testing can all affect the outcome of the experiment. There are several studies showing increased HPA reactivity lasting into adulthood in rodents that experienced stress during adolescence. Isolation housing for three weeks during adolescence prolonged the corticosterone release in response to a stressor when tested two weeks following isolation (Lukkes et al., 2009). Restraint for 10 days at one hour a day during adolescence, increased basal corticosterone levels measured two days after the stressor (Lepsch et al., 2005). One experiment presented restraint, forced swim, and ether stress, once each during adolescence, which increased basal corticosterone levels, an effect still observed seven days after the last stressor (Uys et al, 2006a; Uys et al., 2006b). Chronic unpredictable stress presented for four weeks during adolescence increased basal corticosterone when tested 103 days after the stressor (Pohl et al., 2007). Changing rats' cage partners for 50 days during adolescence increased arginine vasopressin

mRNA levels in the paraventricular nucleus of the hypothalamus, but not adrenocorticotropic hormone or corticotropin releasing hormone mRNA in the paraventricular nucleus of the hypothalamus (Sterlemann et al., 2008). These studies show that several different types of stressors with various schedules when presented during adolescence can increase HPA reactivity long after the original stressor was experienced.

Nevertheless, there are several studies that show decreased HPA activation in rats that have experienced stress during adolescence. Chronic unpredictable stress presented for 42 days during adolescence induced a blunted adrenocorticotropic hormone release to either corticotropin releasing hormone injection or three minutes of ether exposure (Goliszek et al., 1996). Alternating cage partners for 50 days during adolescence decreased basal levels of ACTH when measured in the morning but not in the afternoon (Schmidt et al., 2007). Chronic unpredictable stress of a mild nature presented for four weeks during adolescence decreased basal levels of corticosterone when measured in the evening two weeks later (Toth et al., 2008). Forced swim, restraint and ether stress presented once each during adolescence decreased the number of cells containing GRs in the dentate gyrus of the hippocampus (Uys et al., 2006b). Changing cage partners for 50 days during adolescence decreased the levels of MR and GR mRNA found in the CA1 region but not the dentate gyrus of the hippocampus (Sterlemann et al. 2008). These studies show that stress during adolescence can cause a dampened HPA response to stress, lower baseline levels of HPA activity, and decreased levels of receptors to respond to corticosterone. However, these effects are potentially compensatory responses to heightened levels of corticosterone from the stressors. For example lowered basal levels of corticosterone may be the result of increased negative feedback induced by the repeated stressor presentations. Negative

feedback can reduce the production of corticosterone in the adrenals, and thus the rats would display lower basal levels.

There is a set of studies that found no lasting effect of stress experienced during adolescence on the HPA axis. Three sessions of foot shock that were two days apart during adolescence had no effect on corticosterone response to foot shock when tested two months later (Overmier & Murison, 1991). This study presents a problem because the stress used to test the HPA was the same as the stress originally presented, and therefore it is unclear whether there would have been an effect on the HPA if tested with a heterotypic stressor. Chronic isolation for 16 days paired with changing cage partners (McCormick et al., 2005, Mathews et al., 2008; McCormick et al., 2008), chronic unpredictable stress for 11 days (Maslova et al., 2002), exposure to predator odor for 25 minutes a day for three days (Toledo-Rodriguez & Sandi, 2007), exposure to predator odor on five separate occasions for 30 minutes (Wright et al., 2008), had no effect on corticosterone response to a new stressor when tested in adulthood. Sleep deprivation that prevented rapid eye movement (REM) sleep for one week during adolescence had no lasting effect on basal levels of corticosterone (Shaffery et al., 2006). Taken together, these studies show that not all types and schedules of stressors have a lasting effect on HPA activity. It is possible that the difference between these groups of studies lies in the level of corticosterone released over the course of the stressor presentation. Several of the studies that do observe long term effects include stressor presentations of much longer duration than those that do not. Adolescent rats have been shown to habituate their corticosterone release over the course of 16 days of chronic isolation and to a lesser extent when that isolation is paired with changing the cage partner upon return to the home cage (McCormick et al., 2007). Therefore different levels of exposure to corticosterone may also be the reason for the discrepancy between studies

in lasting effects on the HPA. Further research is required to determine the precise role of corticosterone exposure during adolescence on the ongoing development of the HPA axis.

Adolescence, Stressors, Anxiety and Depressive Behavior

Stressors, both acute and repeated, can also affect the behavior of adolescents. There is evidence that stress during adolescence can increase anxiety as measured in the EPM. In mice, exposures to an adult male for five minutes a day from postnatal day 28 through 32, increased anxiety on the EPM at day 33, but the effect was not observed when one hour of restraint was presented daily from postnatal day 28 through 32 and mice were tested in the EPM at day 33 (Stone & Quartermain, 1997). However, neither five days of restraint nor isolation for 90 minutes a day during adolescence had an effect on anxiety in the EPM when rats were tested during adolescence (Doremus et al., 2009b). It may be that changes in anxiety sometimes do not surface until the rat matures.

Other studies find that these increases in anxiety exist when the subjects are tested long after the cessation of the stressor. Chronic isolation, when it was coupled with the social stress of changing cage partners daily for 16 days, increased anxiety when rats were tested as adults at postnatal day 70, but not when tested during adolescence at postnatal day 45 (McCormick et al., 2008). Changing cage partners, a social stressor in adolescent mice, increased anxiety on the EPM when the mice were tested as adults (Schmidt et al., 2007). Exposure to predator odor once during adolescence, at day 28, increased anxiety on the EPM as well as in an acoustic startle test of anxiety when rats were tested as adults, nine weeks old (Tsoory et al., 2007). Alternating cage partners twice weekly for seven weeks also increased anxiety in adulthood, 12 months after the stressor (Sterlemann et al., 2008). Chronic unpredictable stress during adolescence, from postnatal day 23 to 51, reduced anxiety on the EPM in adulthood, postnatal day 72, when the

stressors were severe, such as foot shock, but not with more mild stressors like a cage tilt (Pohl et al., 2007).

The effect of stress during adolescence on depressive behavior is less well studied than anxiety, and the few studies there are published present conflicting results. Housing animals in isolation during adolescence increased depressive behavior in the second session of the FST when tested during adolescence (Leussis et al., 2008). Chronic unpredictable stress for four weeks during adolescence had no effect on behavior in the FST when measured in adolescence (Toth et al., 2008). Chronic isolation paired with changing cage partners for 16 days during adolescence did not affect behavior in the FST when rats were tested as adults, postnatal day 70 (Mathews et al., 2008). It is possible that those studies showing no effect of stress on the FST may be a result of the rats habituating their corticosterone release to the stressor, considering direct administration of corticosterone increases depressive behavior in adults (Kalynchuk et al., 2004; Gregus et al., 2005; Johnson et al., 2006).

Comparable to the research performed with adults, stress during adolescence alters structure of the brain areas linked to the affected behaviors previously discussed. Chronic unpredictable stress of both physical and social nature presented for 28 days during adolescence decreased hippocampal volume when measured three weeks following the stressor (Isgor et al., 2004). Swim, restraint and ether stress each presented once on different days during adolescence decreased levels of growth promoting proteins in the hippocampus (Uys et al., 2006a). However, chronic unpredictable stress with mild stressors presented for four weeks during adolescence increased levels of growth promoting protein in the hippocampus and increased neurogenesis in the hippocampus when measured during adulthood (Toth et al., 2008). Five days of isolation during adolescence decreased spinophilin, which is a protein found in dendritic spines, in the

basolateral amygdala and the central nucleus of the amygdala when measured during adolescence (Leussis et al., 2008). The effect of stress on these structures is not as well studied in adolescence as in adults. Stress during adolescence has similar effects on behavior to stress experienced during adulthood, but those effects are enduring when the stressor is experienced during adolescence. Further study is still required to clarify the mechanisms of these enduring effects.

The Present Study

The above literature illustrates that in adults, exposure to stressors or to elevations in corticosterone can increase anxiety-like and depressive behavior, but these effects are not as clear when the stressor is administered during adolescence. This is partly because a wide variety of stressors have been used, and therefore it is difficult to compare results from study to study. Variations in individual responses to stressors and ability to habituate to stressors also make studies of chronic stressors difficult to interpret. Sterner and Kalynchuk (2010) argue that administering corticosterone in a uniform dose provides a more consistent effect with less variation due to individual differences. When presenting rats with a stressor individual rats will respond with different amounts of corticosterone, and each rat will habituate to the stressor at a different time. These issues do not exist in an experiment that gives a consistent dose of corticosterone to each rat. Therefore experiments that involve the administration of corticosterone are more likely to yield conclusive results than those that use an arbitrarily chosen stressor. Several studies have shown corticosterone to increase depressive behavior in the FST (Kalynchuk et al., 2004; Gregus et al., 2005 Johnson et al., 2006; Murray et al., 2008; Marks et al., 2009; Lee et al., 2009; Gourley & Taylor, 2009). Additionally, corticosterone administration has been shown to produce the same effects on volume of brain regions that is observed in

patients with depression, decreased hippocampal and prefrontal cortex volume and increased amygdala volume (reviewed in Sterner & Kalynchuk, 2010). However, the effects of corticosterone administration during adolescence have not been studied.

The purpose of the present study is to determine the effect of corticosterone administration during adolescence on the HPA axis and on anxiety-like and depressive behavior as measured by the EPM and FST respectively. Based on the other research in this area, corticosterone is expected to increase anxiety-like and depressive behaviors, just as corticosterone does in adults. However, it is predicted that exposure during adolescence will produce greater and longer lasting effects as compared to exposure during adulthood. It is expected that exposure to corticosterone during adolescence will cause increased release of corticosterone in response to a stressor, similar to that observed with social stressors (Mathews et al., 2008), and that this altered HPA response will be enduring. It is also predicted that this HPA effect will be longer lasting in rats exposed to corticosterone during adolescence than in those exposed during adulthood.

Methods: Experiment 1

Animals

112 male Long-Evans rats, obtained from Charles River, St. Constant, Quebec, arrived in the colony, half were 22 days of age, adolescents, and half were 64 days of age, adults. They were housed in pairs in plastic (polycarbonate) cages and were provided with a plastic tube in each cage for enrichment throughout the experiment. Rats were identified by tail coloring with a felt tip marker. The rats were kept on a 12 hour light, 12 hour dark light cycle, and were given unlimited access to rat chow and water. Rats were given seven days to adjust to these housing conditions before the experimental procedures. All experimental procedures were consistent with National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), and Canadian Council on Animal Care guidelines and were approved by the Brock University Institutional Animal Care and Use Committee.

Treatment

Starting on postnatal day 30 for the adolescents and 70 for the adults, each rat received one subcutaneous injection daily for 16 days, with the last injection given on postnatal day 45 or 85. Half of the rats in each age group (n = 28 for each age) received vehicle (VEH) injections consisting of isotonic saline and 2% Tween-80 (purchased from Sigma-Aldrich, St. Louis, MO), and the other half (n = 28 for each age) received 40 mg / kg / mL corticosterone (CORT; purchased from Steraloids, Newport, RI; procedure as in Kalynchuk et al., 2004; Sousa et al., 1998) suspended in the aforementioned solution. After injection the rat was immediately returned to the home cage, and each pair of rats that shared a cage were administered the same treatment. Previous research has found this dose of corticosterone injection to produce plasma corticosterone levels of approximately 2100 ng/mL one to four hours after the injection (Sousa et

al., 1998). The rats were weighed once every three days, and the injection volume was consequently adjusted. After the injection period rats were weighed once a week for the remainder of the experiment.

Home cage behavior

Home cage behavior was monitored to determine the effects of corticosterone treatment on social behavior. Monitoring began on the third day, and continued for a total of eight times for the adolescent group and five times for the adult group throughout the administration of injections. Ten minutes after injection, the rats were monitored while in their cages for 45 minutes to determine if the injection type affected social interaction. A time sampling method was used, as described in McCormick et al. (2007), whereby the behavior of each rat was recorded every three minutes for a total of 14 times per session. The behaviors were collapsed into the following categories associative (sniffing, following, allogrooming, mutual circling, pushing, play soliciting, playing, and lying down together) or non-associative (inactive, exploring, self-grooming, eating, and drinking). The adolescents were observed eight times, and the adults were observed five times. The total occurrences of each behavior in a session were tallied, and the percentage of behaviors observed that were associative was calculated for each day behavior was observed.

Elevated Plus Maze

The elevated plus maze (EPM) was made of four arms, two open and two closed, constructed out of grey painted plywood. The arms were 50 cm in length, and the walls enclosing the closed arm were 42 cm high. The sides of the open arm had a ledge that is 1.3 cm high. The maze was raised 78.74 cm off the ground, and was kept in a separate room from the colony. During testing the maze was illuminated with dim white light, and white noise (~60 dB) was

presented. Each rat was placed in the closed arm at the end farthest from the center and was allowed to explore the maze for five minutes. All sessions were video taped, and tapes were later scored while blind to condition of the animal being tested. Tapes were scored for time spent in the open arm, closed arm and center as well as the total number of rears (when the rat stands on its hind legs) and head dips (when the rat reaches its head over the edge of the open arm). Half of each the vehicle and the corticosterone groups at each age were tested on the EPM one day after their last injection, on postnatal day 46 or 86 (n = 14 for both VEH and CORT groups at each age). The remaining rats were tested on the EPM 25 days after the last injection, at postnatal day 70 or 110 (n = 14 for both VEH and CORT groups at each age).

Forced Swim Test

The forced swim test (FST) consisted of a clear cylinder 20.3 cm in diameter filled 24.1 cm high with water such that a rat was unable to touch the bottom or climb over the side. The water was warmed to 26 °C, and it was changed for every rat. Rats were placed in the water and left in the tank for twenty minutes. All sessions were videotaped, and tapes were later scored while blind to condition of the animal being tested. Tapes were scored for time spent immobile, swimming, diving and climbing. Of the 56 rats tested in the EPM immediately after the injections, 32 rats were tested with the FST the next day, postnatal day 47 or 87 (n = 8 for adolescent VEH, adolescent CORT, adult VEH, and adult CORT). Of the 56 rats that were tested in the EPM, 25 days after the last injection, 32 rats were tested with the FST the next day, postnatal day 71 or 111 (n = 8 for adolescent VEH, adolescent CORT, adult VEH, and adult CORT).

FST Methodology

Classically the FST is administered in two sessions that are 24 hours apart, as described in the Introduction. An experimental manipulation, for example a dose of antidepressants, would be given between the two sessions, and the result would be observed on the second session (Porsolt et al., 1977; Armario et al., 1988; Detke et al., 1995; Tejani-Butt et al., 2003; Pechnick et al., 2008). However, to determine if a chronic manipulation, for example 21 days of corticosterone injections, could produce a depression like state, the injections would be given and a only single session of the FST would be necessary to show increased depressive state (Kalynchuk et al., 2004; Gregus et al., 2005; Johnson et al., 2006). Antidepressants can also decrease immobility in a one session FST (Overstreet & Griebel, 2004; Overstreet et al., 2004). When compared directly, the effects of chronic corticosterone treatment for 21 days in adults were the same whether tested with a one day version of the test or a two day version (Marks et al., 2009). Therefore, although several of the experiments discussed above employ the two session version of the FST, the experiments described here use a one session test.

Plasma Corticosterone Determination

To measure the stress-induced release of corticosterone in response to forced swim, blood was collected by tail nick from all of the rats directly after, 45 minutes after, and 90 minutes after the forced swim test (n = 8 for VEH day 47 and 87, CORT day 47 and 87, VEH day 71 and 111 and CORT day 71 and 111). Blood samples were centrifuged at 3000 rpm and 4 °C for 20 minutes, and then the plasma was collected and stored at -80 °C until the time of measurement. Plasma corticosterone concentrations were determined using a highly specific corticosterone antiserum (obtained from Dr. Greg Brown, University of Toronto) and [^{3}H] corticosterone (70.0 Ci/mmol, Perkin Elmer, Wellesley, MA) as a tracer. The minimum detection level for the assay is

15 pg per tube. The antiserum cross-reactivity is less than 1% with cortisol, testosterone, and estradiol. Intra- and inter-assay reliability is less than 5% and 10% respectively. All samples for this experiment were measured at the same time.

Statistical Analysis

To facilitate interpretation given the number of variables and the predicted interactions, the administered in adolescence versus administered in adulthood groups were analyzed separately. Furthermore, the focus of the investigation was the effect of corticosterone treatment at each age, and age differences would be expected for most of the measures. Weight differences were analyzed using a repeated measures analysis of variance (ANOVA) with the between subject factor of treatment, and where appropriate these were followed up with t-tests. In cage behavior was analyzed with repeated measures ANOVA with treatment as a between subjects factor. Behavioral measures during the EPM were analyzed using multivariate analysis of variance (MANOVA) with between subject factors of treatment (corticosterone or vehicle) and timing of testing [soon after (Immediate groups) or weeks after (Enduring groups) treatment). When appropriate, these were followed up with MANOVAs for each of the different timing of testing groups with the between subject factor of treatment. The plasma concentrations of corticosterone were analyzed using a repeated measures ANOVA with treatment and timing of testing as the between subject factors. This was followed up with separate repeated measures ANOVAs for each timing of testing group. Fisher's protected least square differences were used for post hoc analyses where appropriate. An alpha level of $p < 0.05$ was considered significant.

Table 1.

Sample sizes of groups for each measure in Experiment 1.

	Adolescents				Adults			
	Immediate		Enduring		Immediate		Enduring	
Treatment	CORT	VEH	CORT	VEH	CORT	VEH	CORT	VEH
Weight	14	14	14	14	14	14	14	14
Behavior[1]	9	9	-	-	9	9		
EPM	13[2]	13[2]	14	14	14	14	12[2]	14
FST	8	9[2,3]	8	6[2]	8	8	6[6]	8
Cort after FST[5]	4	3	4	4	2	1	3	2

1. Behavior was monitored in a chosen subset of cages during injection period; therefore rats were not differentiated between immediate and enduring. N is the number of cages monitored, each of which housed two rats.
2. Data were lost after testing because of equipment failure or malfunction, thereby reducing the sample size by one.
3. Two rats, that were treated with vehicle injections but not intended for use in the FST study, were accidentally tested in the FST, and therefore their data were included in the analysis.
4. A pair of rats had body weights that were too low after corticosterone (cort) injections to undergo the FST because they were no longer buoyant.
5. Corticosterone levels were only analyzed for those rats from which we were able to collect a usable sample at all three time points.
6. Data were lost after testing because of equipment malfunction, thereby reducing the sample size by two.

Results: Experiment 1

In all data analyses, adolescents and adults are analyzed separately. Additional statistical analyses can be found in Appendix A.

Weight

A repeated measures ANOVA of weight for the adolescents with treatment as the between subjects factor revealed a significant two way interaction between time point and treatment, $F(5,270) = 87.34$, $p < 0.001$. Post hoc independent samples t-tests indicated that the treatment groups did not differ in weight, $p = 0.92$, on the first day of treatment, and on all other days, the corticosterone treated rats weighed less than the vehicle treated rats, $p < 0.001$ for all days, (see Figure 1).

A repeated measures ANOVA of weight for the adults with treatment as the between subjects factor showed a significant two way interaction between time point and treatment, $F(5,270) = 251.07$, $p < 0.001$, such that vehicle treated rats were gaining weight, and corticosterone treated rats were losing weight over the duration of treatment. Post hoc independent samples t-test indicated that there was no difference between the treatment groups on day one, $p = 0.50$. On every other day weight was measured, corticosterone treated rats weighed less than vehicle treated rats, $p < 0.001$ for all days (see Figure 1).

For the adolescents that were tested several weeks after treatment, a repeated measures ANOVA of weight each week after the administration of the injections indicated a significant interaction between time point and treatment, $F(2,52) = 4.04$, $p = 0.02$. Post hoc t-tests indicated that the corticosterone treated rats weighed significantly less than the vehicle treated rats, $p < 0.001$ at each time points (see Figure 1).

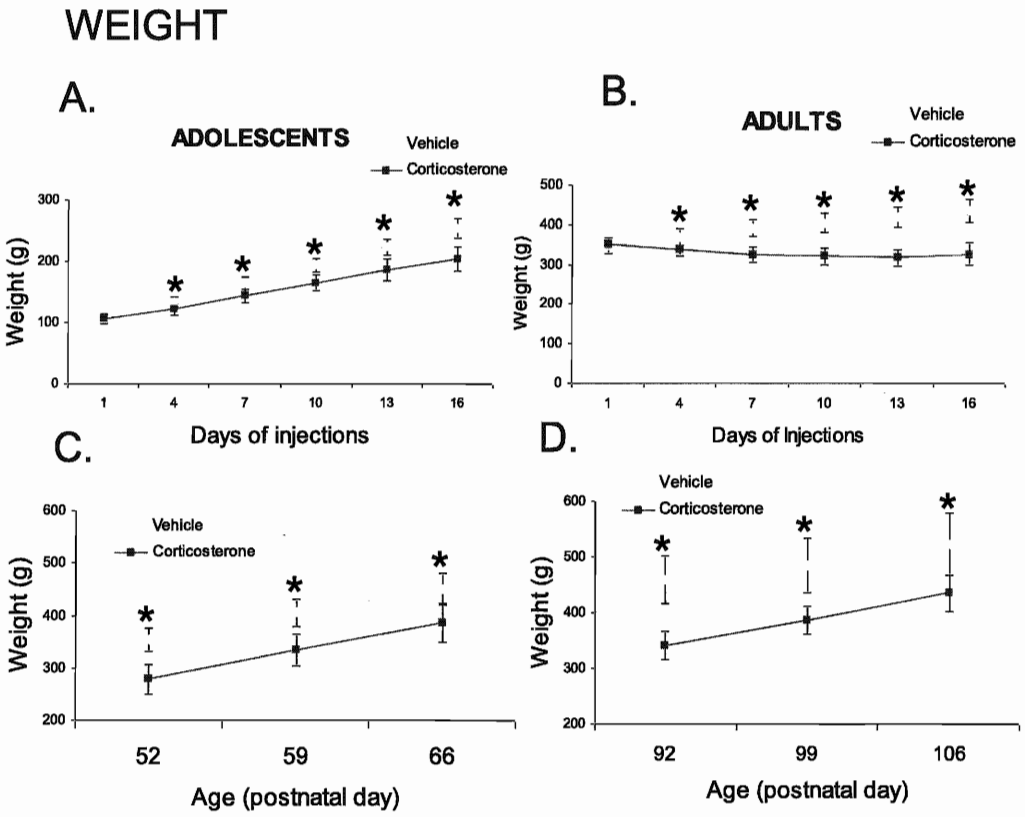


Figure 1. Left column: mean of the weight of the adolescents A. during treatment, and C. after treatment. Right column: mean of the weight of the adults B. during treatment and D. after treatment. Error bars represent the standard error of the mean (SEM). * Indicates time points at which the corticosterone treated rats weigh less than the vehicle treated rats, $p < 0.001$.

For the adults that were tested several weeks after treatment, a repeated measures ANOVA of weight each week after the administration of the injections indicated a significant interaction between time point and treatment, $F(2,52) = 22.03$, $p < 0.001$. Post hoc t-tests indicated that the corticosterone treated rats weighed less than the vehicle treated rats, $p < 0.001$, at each time point (see Figure 1).

Home Cage Behavior

A repeated measures ANOVA of the percent of the behavioral observations among adolescents in which cage partners were associating with one another found a significant main effect of the day of observation, $F(7,112) = 4.18$, $p < 0.001$ (see Figure 2). There was no main effect of treatment, $F(1,16) = 0.12$, $p = 0.74$, and there was no interaction between treatment and day of observation, $F(7,112) = 1.11$, $p = 0.36$.

A repeated measures ANOVA of percent of the behavioral observations among adults found that percentage of incidents in which cage partners were associating with one another differed from day to day of observation, $F(4,64) = 5.50$, $p = 0.001$ (see Figure 2). There was no main effect of treatment, $F(1,16) = 0.003$, $p = 0.87$, and there was no interaction between treatment and day of observation, $F(4,64) = 1.04$, $p = 0.39$.

IN CAGE BEHAVIOR

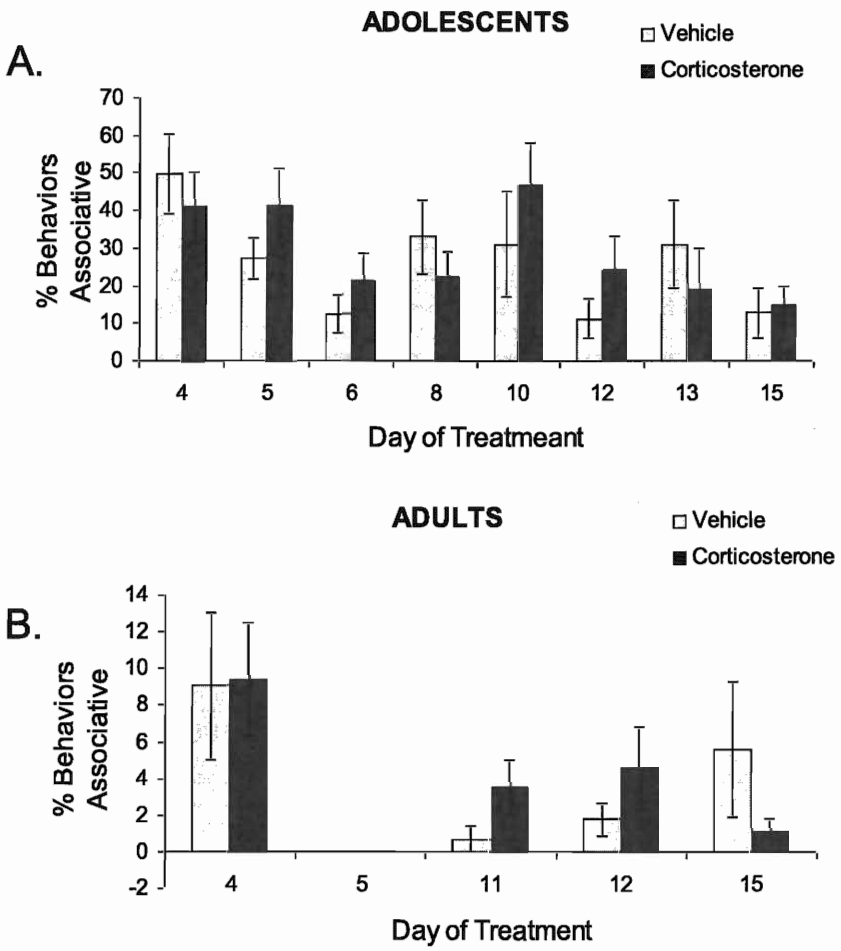


Figure 2. The mean of the percent of behaviors monitored in the home cage that were associative during treatment for A. adolescents and B. adults. Error bars represent the standard error of the mean (SEM).

Elevated Plus Maze

All variables analyzed were checked to determine if there were correlations between the variables analyzed, and several variables are correlated with one another (see Table 2 and Table 3). A MANOVA of time in the open arm, percent of entries to the open arm, time in the center, closed arm entries, head dips, rears, and latency to the open arm with treatment and time of testing as the between subjects factors, showed that, for rats treated in adolescence, there was a significant effect of time of testing on several variables in the EPM. Irrespective of type of treatment, rats treated in adolescence and tested several weeks after treatment demonstrated more time in the open arm, $F(1,46) = 27.66$, $p < 0.001$, a lower latency to reach the open arm, $F(1,46) = 3.92$, $p = 0.05$, a higher percent of entries to the open arm, $F(1,46) = 26.61$, $p < 0.001$, more head dips, $F(1,46) = 12.58$, $p = 0.001$, and more rears, $F(1,46) = 26.15$, $p < 0.001$, than did adolescents tested immediately after treatment, (see Figure 3). Rats treated in adolescence and tested several weeks later also spent more time in the center, $F(1,46) = 9.76$, $p = 0.003$, and they made more entries to the closed arm, $F(1,46) = 4.86$, $p = 0.03$, than did rats tested soon after treatment ended (see Figure 4). There were no significant effects of corticosterone treatment or interactions between time of testing and treatment, see Appendix A for details.

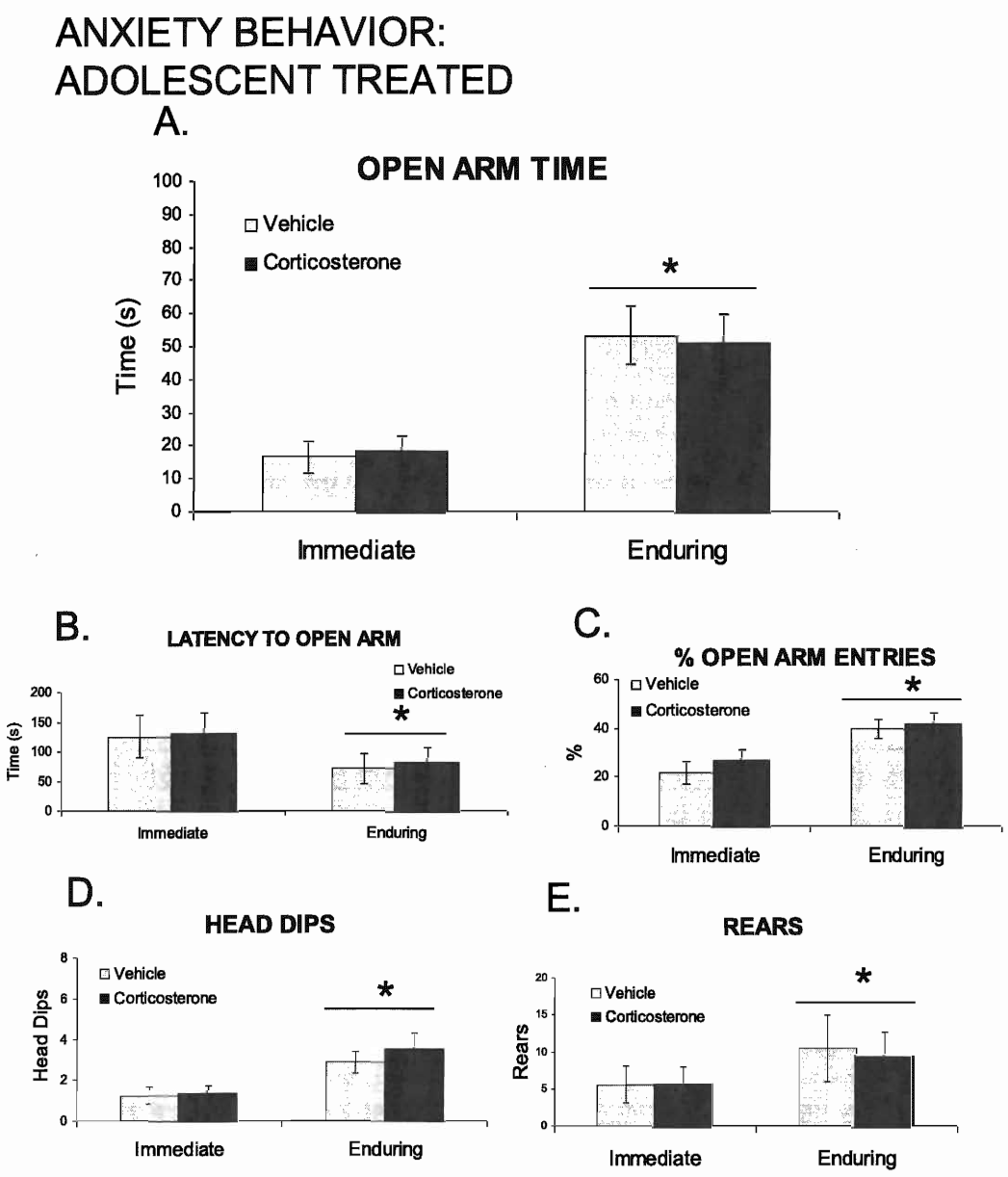
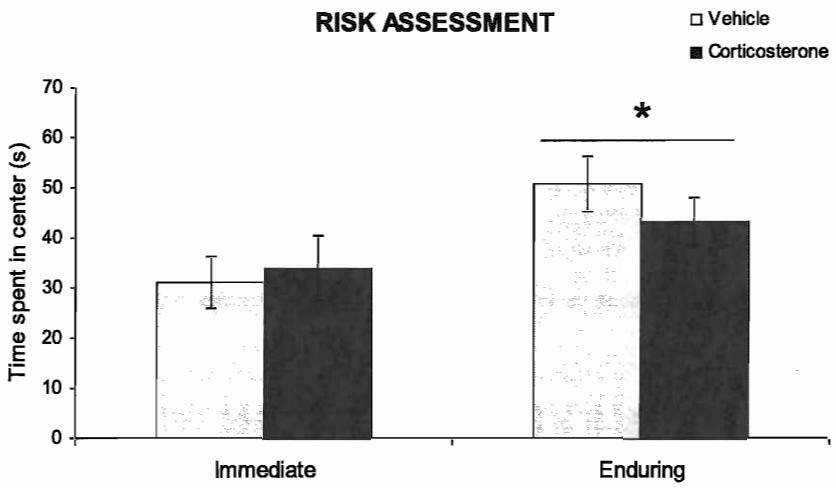


Figure 3. Means for anxiety related behaviors on the EPM in adolescent treated rats: A. time spent in the open arm, B. latency to reach the open arm, C. percent entries to the open arm, D. head dips, and E. rears. Error bars represent the standard error of the mean (SEM). * Indicates a main effect of time of testing, p = 0.05 or less.

NON-ANXIETY BEHAVIORS: ADOLESCENT TREATED

A.



B.

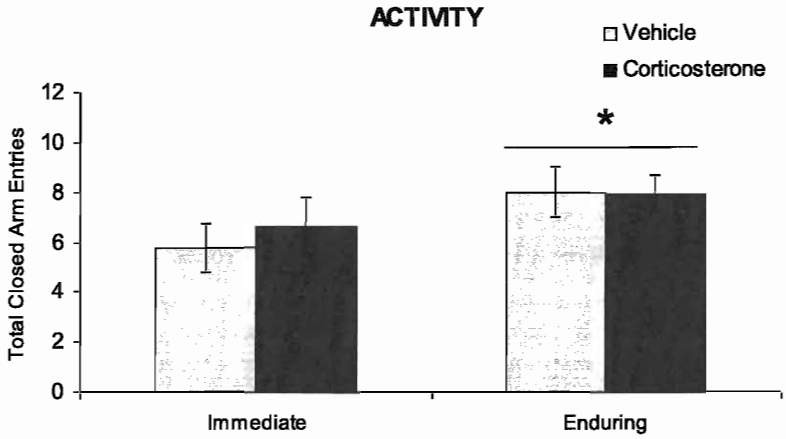


Figure 4. The means for behaviors performed by the adolescents on the EPM: A. time spent in the center of the maze, which is associated with risk assessment, and B. total entries to the closed arm, which is associated with activity. Error bars represent the SEM. * Indicates a main effect of time of testing, $p = 0.05$ or less.

Table 2.

Correlations of EPM Variables Grouped by Related Construct for Adolescent Corticosterone or Vehicle Treated Rats

	Anxiety					Risk	Activity
	Time open	% open entries	Latency to open	Head dips	Rears	Time center	Closed entries
Time open	-	0.67*	-0.67*	0.84*	0.60*	0.62*	0.68*
% open entries		-	-0.65*	0.65*	0.42*	0.58*	0.46*
Latency to open			-	-0.62*	-0.42*	-0.77*	-0.80*
Head dips				-	0.47*	0.51*	0.49*
Rears					-	0.47*	0.44*
Time center						-	0.77*

* indicate statistically significant correlations, $p = 0.05$ or smaller.

For rats treated as adults, a MANOVA of time in the open arm, percent of entries that are to the open arm, time in the center, closed arm entries, head dips, rears and latency to the open arm for rats treated as adults indicated that there was a significant interaction between treatment and time of testing on head dips, $F(1,49) = 7.45$, $p = 0.009$, and closed arm entries, $F(1,49) = 15.16$, $p < 0.001$, and a near significant interaction between time of testing and treatment on time in the open arm, $F(1,49) = 3.41$, $p = 0.07$. Additional details are available in Appendix A. This analysis was followed up with a MANOVA for each time of testing group separately.

For those adults tested immediately after treatment, corticosterone treated rats entered the closed arms fewer times than did vehicle treated rats, $F(1,26) = 7.01$, $p = 0.01$. For those rats treated as adults and tested several weeks later, corticosterone treated rats entered the closed arm more often than did vehicle treated rats, $F(1,25) = 8.73$, $p = 0.007$ (see Figure 6). The rats treated with corticosterone as adults and tested several weeks later also performed more head dips than

did the corresponding vehicle treated rats, $F(1,25) = 10.99$, $p = 0.003$. There was a near significant trend for the rats treated with corticosterone as adults and tested several weeks later to spend more time in the open arm than those treated with vehicle, $F(1,25) = 3.73$, $p = 0.07$ (see Figure 5).

Table 3

Correlations of EPM Variables Grouped by Related Construct for Adult Corticosterone or Vehicle Treated Rats

	Anxiety					Risk	Activity
	Time open	% open entries	Latency to open	Head dips	Rears	Time center	Closed entries
Time open	-	0.51*	-0.60*	0.76*	0.25	0.15	0.43
% open entries		-	-0.74*	0.36*	0.28*	0.46*	0.13
Latency to open			-	-0.50*	-0.35*	-0.56*	-0.62*
Head dips				-	0.31*	0.04	0.37*
Rears					-	0.19	0.28*
Time center						-	0.53*

* indicate statistically significant correlations, $p = 0.05$ or smaller.

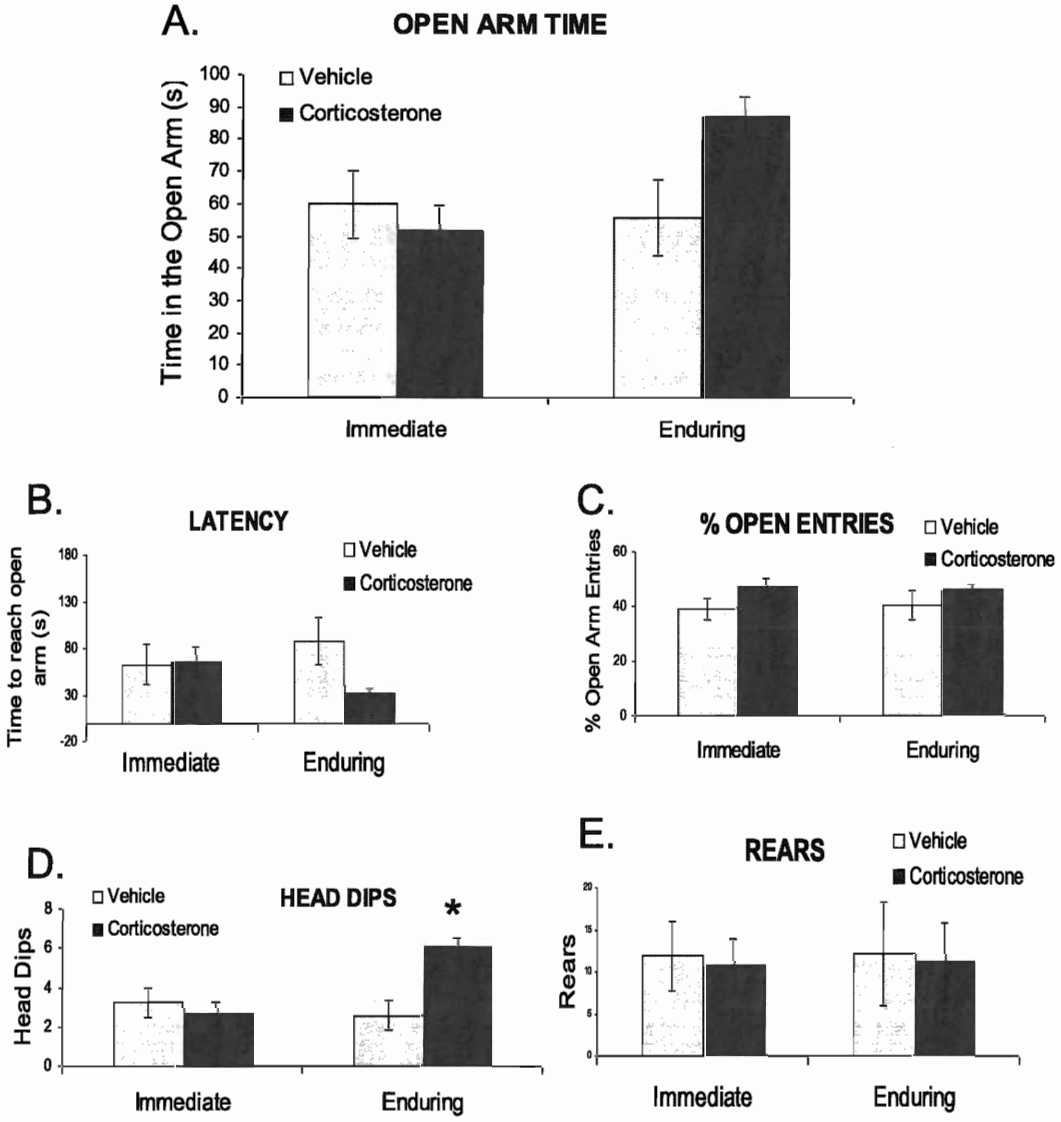


Figure 5. Means of anxiety related behaviors on the EPM for adult treated rats: A. time spent in the open arm, B. latency to reach the open arm, C. percent entries to the open arm, D. head dips, and E. rears. Error bars represent the SEM. * Indicates a significantly different from corresponding vehicle group, $p = 0.003$ or less.

NON-ANXIETY BEHAVIOR
ADULT TREATED

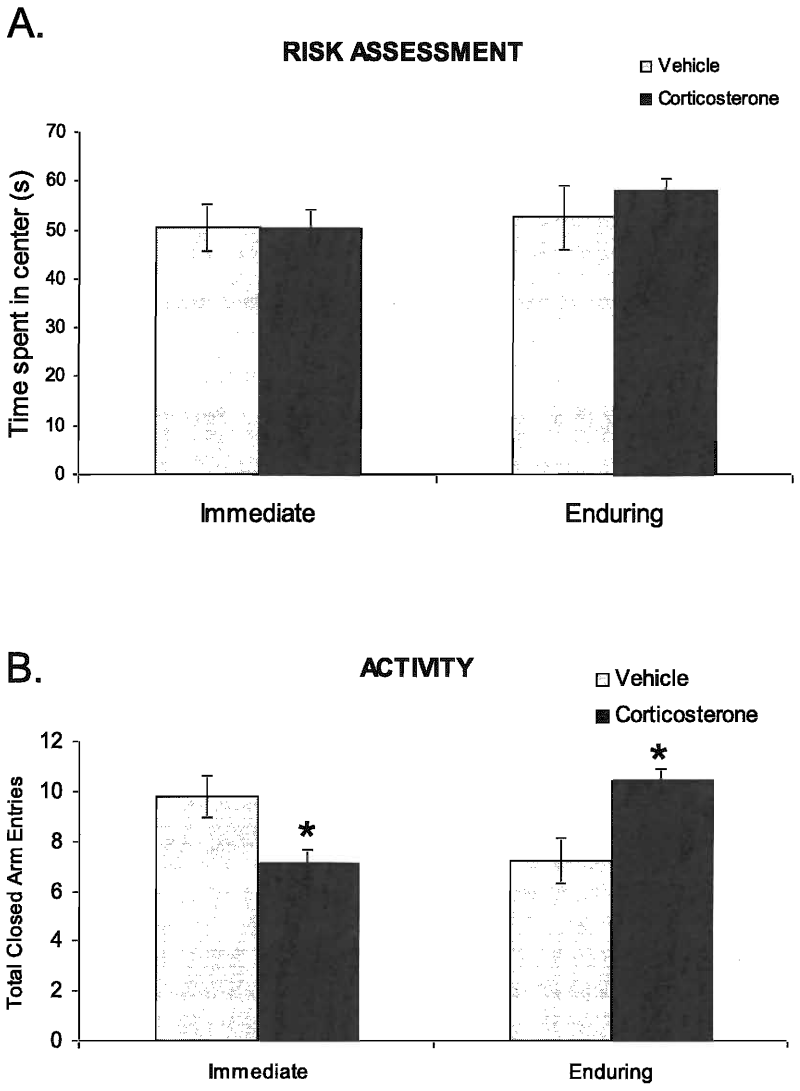


Figure 6. Means of behaviors performed by the adults on the EPM A. time spent in the center of the maze, which is associated with risk assessment, and B. total entries to the closed arm, which is associated with activity. Error bars represent the SEM. * Indicates where the corticosterone group is significantly different from the corresponding vehicle group, $p = 0.01$.

Forced Swim Test

Latency to Immobility: For rats treated with corticosterone or vehicle in adolescence, an ANOVA of latency to immobility showed that those tested immediately after treatment had a greater latency to immobility than those tested several weeks later, $F(1,27) = 16.04$, $p < 0.001$. For rats treated in adulthood, an ANOVA of latency to immobility found no significant effects of timing of testing or of treatment and no interaction between the two factors (see Figure 7).

LATENCY TO IMMOBILITY

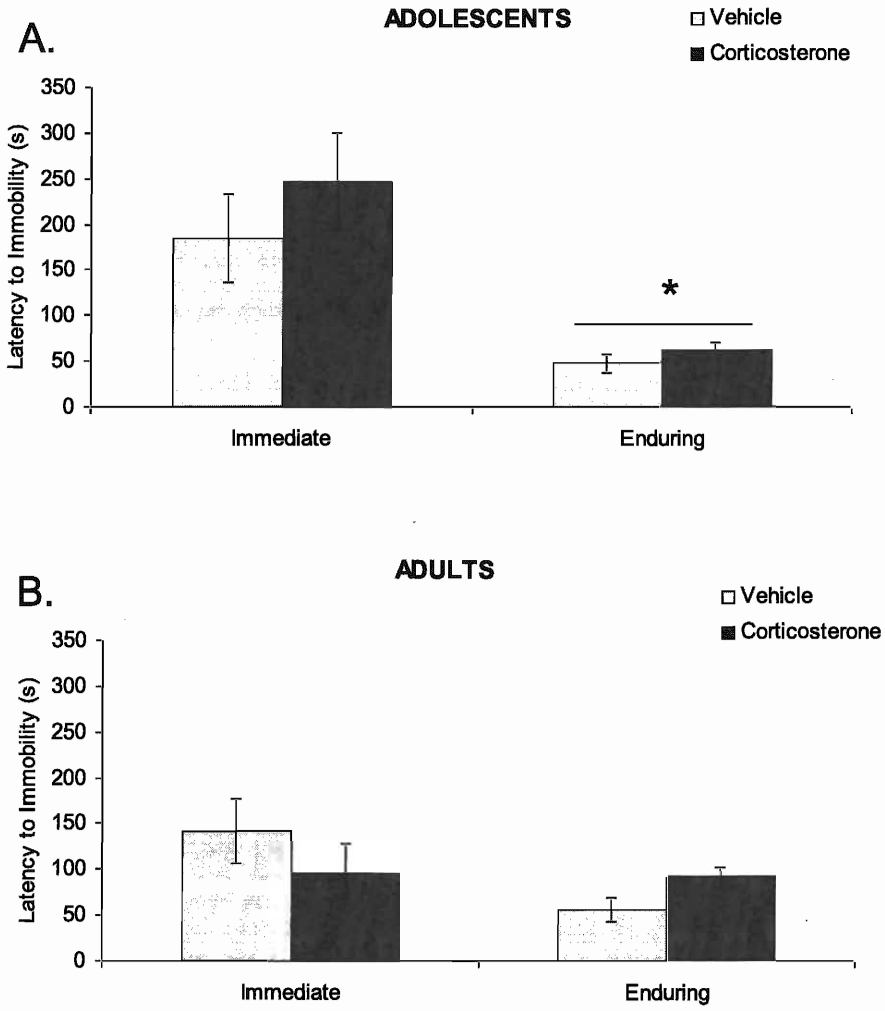


Figure 7. The means for the amount of time in the FST before spending a minimum of five seconds immobile. Error bars represent the SEM. * Indicates where the time of testing group was significantly different, $p < 0.001$.

Duration of Immobility: For rats treated in adolescence there was a time point by treatment interaction $F(3,81) = 2.73$, $p = 0.05$. Additionally, rats treated in adolescence and tested several weeks later spent more time immobile than did rats tested immediately after treatment, $F(1,27) = 52.0$, $p < 0.001$. For both types of treatment there was a significant effect of time point, $F(3,42) = 11.95$, $p < 0.001$ for vehicle treated rats and $F(3,45) = 43.67$, $p < 0.001$ for corticosterone treated rats. This was followed with paired samples t-tests, which indicate that for as the rats spent more time immobile in the first five minutes of the test than any other time block for both rats treated with vehicle, $p = 0.001$, and corticosterone, $p < 0.001$. For the rats treated with corticosterone, rats spent more time immobile in the last five minute block of the test than during either the second, $p = 0.007$ or the third five minute block, $p = 0.03$. Overall corticosterone treated rats spent more time immobile as the test progressed, and the vehicle treated rats spent less time immobile in the first five minute block of the test than any other five minute block (see Figures 8 and 9).

For the adults, there also was a significant time point by timing of testing interaction, $F(3,78) = 3.00$, $p = 0.04$. This was followed up with individual t-tests at each time point, which indicated that adults, tested several weeks after treatment, spent more time immobile during the first five minutes of the test than those tested immediately, $p < 0.001$, but were not different at any other time point (see Figure 10 and 11).

ADOLESCENT TREATED AND TESTED IMMEDIATELY

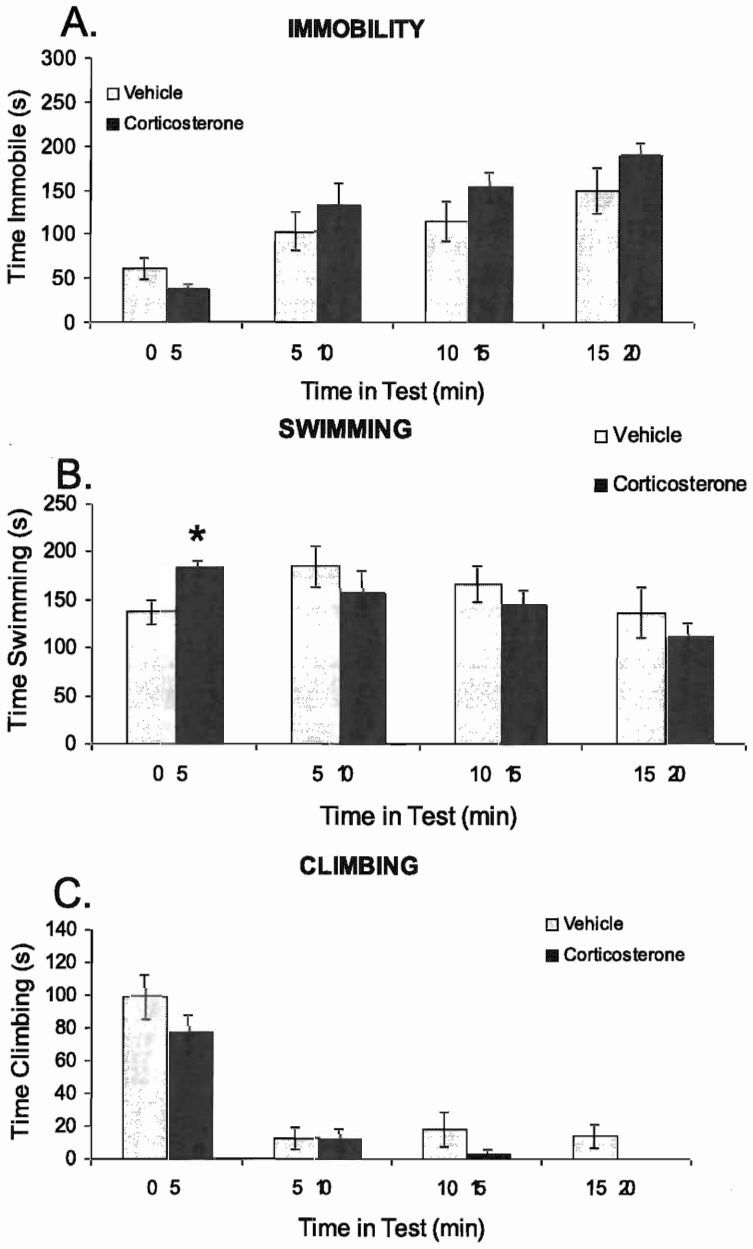


Figure 8. The means for the time the adolescent rats tested for immediate effects spent engaging in the following behaviors in the FST, A. immobility, B. swimming, and C. climbing. Error bars represent the SEM. * Indicates significantly different from corresponding vehicle group, p = 0.01.

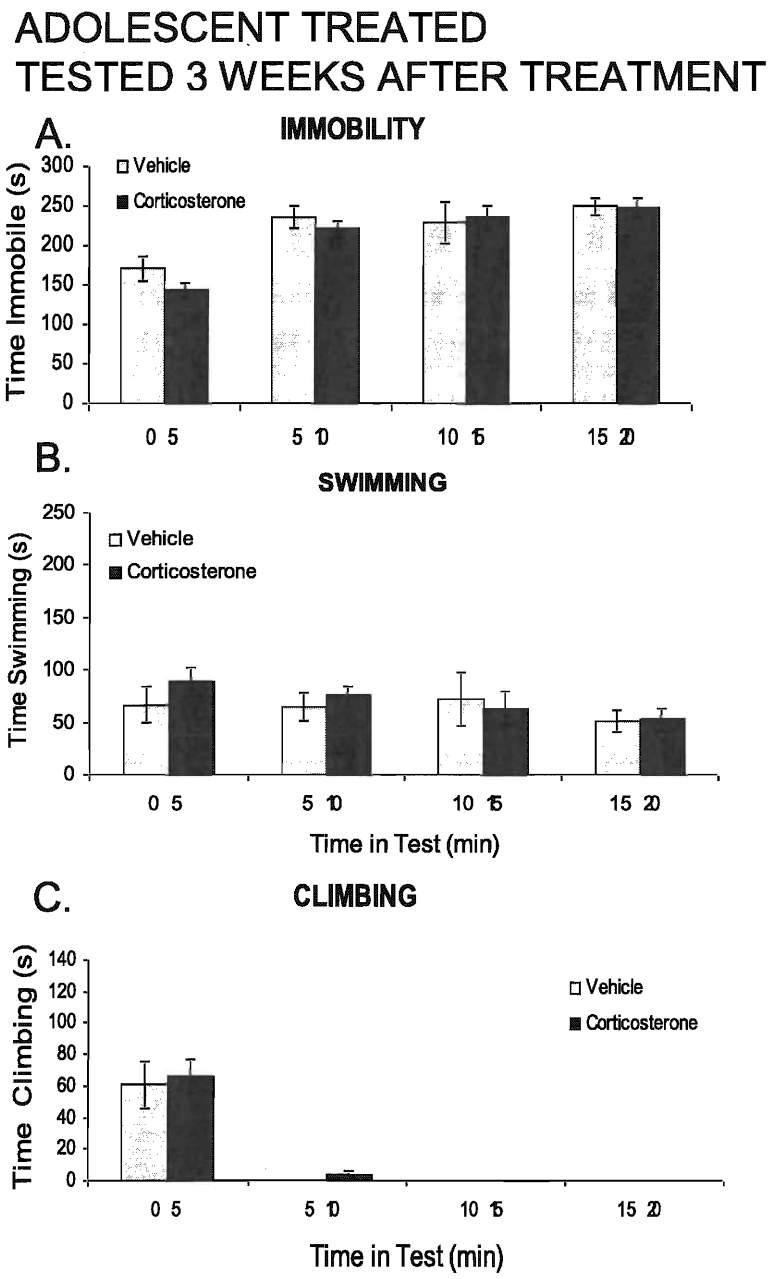


Figure 9. The means for the time the adolescent rats tested three weeks after treatment spent engaging in the following behaviors in the FST, A. immobility, B. swimming, and C. climbing. Error bars represent the SEM.

Duration of Swimming: For the rats treated in adolescence there was a time point by treatment interaction, $F(3,81) = 2.68$, $p = 0.05$. For the rats treated in adolescence and tested immediately there was a significant interaction of time point by treatment, $F(3,45) = 2.87$, $p = 0.05$. This was followed up by an independent sample t-test, which indicated that corticosterone treated rats were swimming more than vehicle treated rats only at the five minute time point, $p = 0.01$ (see Figure 8). For those rats that were treated in adolescence and tested several weeks later there was no effect of time point or treatment (see Figure 9). See Appendix A for additional details.

For the rats treated in adulthood, there was a time point by time of testing interaction, $F(3,78) = 9.93$, $p < 0.001$, and a near significant time of testing by treatment interaction, $F(3,78) = 2.52$, $p = 0.09$. This was followed up with separate repeated measures ANOVAs of time spent swimming for each time of testing group. For the adult treated rats tested immediately after treatment there was a significant effect of time point, $F(3,42) = 7.45$, $p < 0.001$, such that rats swam less as the test progressed, and the same was true for those rats treated as adults and tested several weeks later, $F(3,36) = 3.60$, $p = 0.02$ (see Figures 10 and 11). For those rats treated as adults and tested several weeks later the interaction between time point and treatment approached significance $F(3,36) = 2.15$, $p = 0.11$.

ADULT TREATED AND TESTED IMMEDIATELY

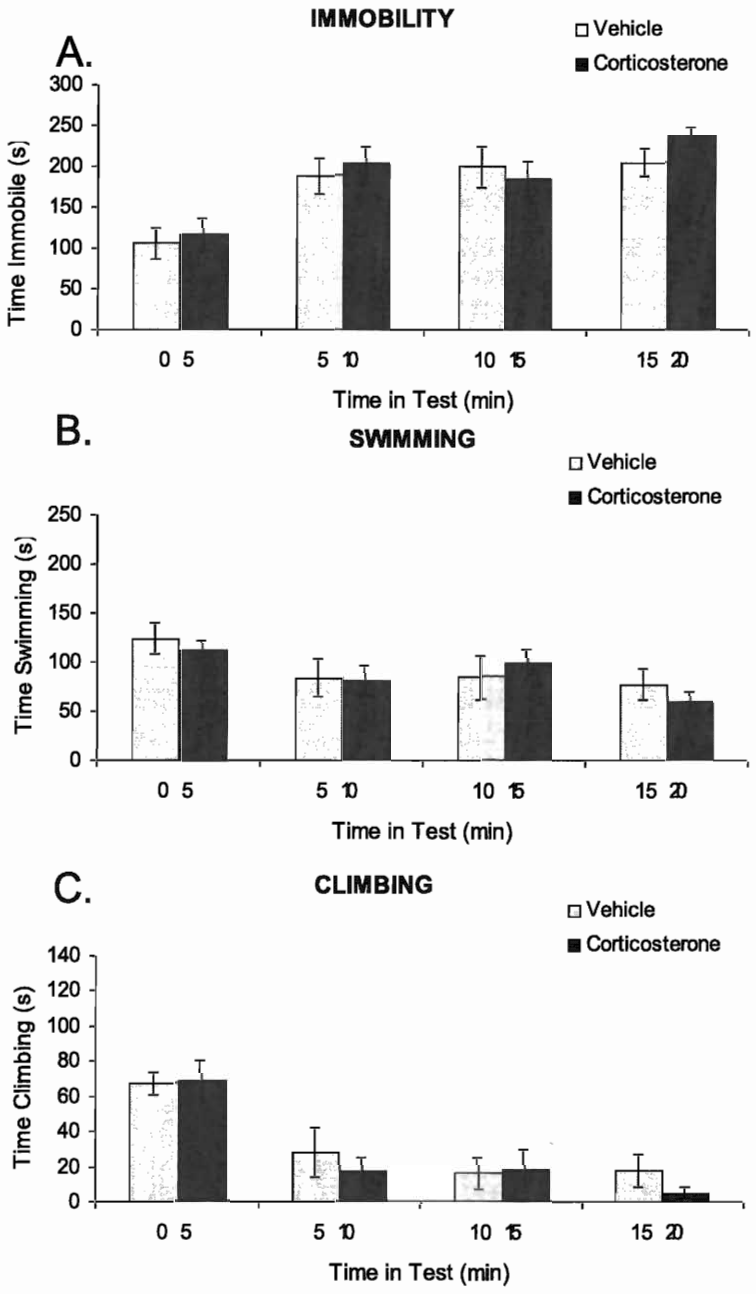


Figure 10. The means for the time the adults rats tested for immediate effects spent engaging in the following behaviors in the FST, A. immobility, B. swimming, and C. climbing. Error bars represent the SEM.

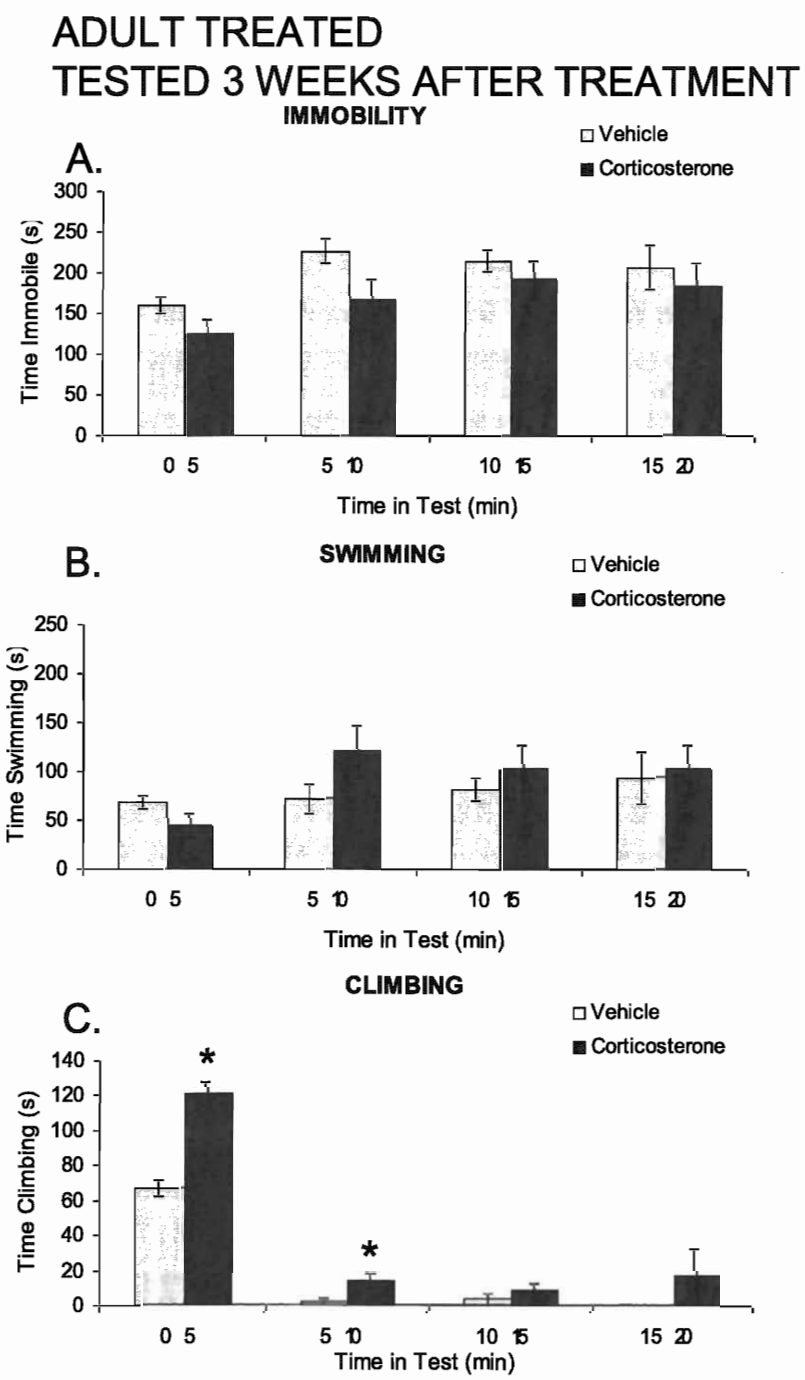


Figure 11. The means for the time the adult rats tested for enduring effects spent engaging in the following behaviors in the FST, A. immobility, B. swimming, and C. climbing. Error bars indicate the SEM. * Indicates significantly different from the corresponding vehicle group, $p = 0.02$ or less.

Duration of Climbing: For the rats treated in adolescence there was a significant effect of time point, $F(3,81) = 100.6$, $p < 0.001$, such that rats climbed less as the test progressed. The rats treated in adolescence and tested several weeks later climbed significantly less than those tested immediately, $F(1,27) = 6.02$, $p = 0.02$. The interaction of treatment and time point approached significance, $F(1,27) = 2.13$, $p = 0.16$.

For rats treated in adulthood the time point by treatment interaction was significant, $F(3,78) = 3.35$, $p = 0.02$, and the time point by time of testing interaction was also significant, $F(3,78) = 6.87$, $p < 0.001$. There was also a significant interaction of treatment by time of testing, $F(1,26) = 5.11$, $p = 0.03$, and a near significant three way interaction $F(3,78) = 2.08$, $p = 0.11$. For those rats treated as adults and tested several weeks later there was a significant time point by treatment interaction, $F(3,36) = 7.40$, $p = 0.001$. This was followed up with an independent t-test showing that the corticosterone treated rats climbed more than the vehicle treated rats at the five and ten minute time points, $p < 0.001$ and $p = 0.02$ respectively (see Figure 11). For those adults tested immediately there was a significant main effect of time point, $F(3,42) = 22.25$, $p < 0.001$, such that rats climbed less as the test progressed (see Figure 10).

Corticosterone after the Forced Swim Test

Because of sampling difficulties, samples were not available for all time points. Thus, only rats with a complete data set across three time points were analyzed. A repeated measured ANOVA of the corticosterone concentration immediately, 30 minutes and 90 minutes after the FST, indicated that for the rats treated in adolescence there was a significant three way interaction of time point by treatment by time of testing, $F(2,44) = 3.33$, $p = 0.05$. For the adolescent treated rats tested immediately, there was a significant main effect of time point, $F(2,20) = 7.11$, $p = 0.005$, such that corticosterone levels decreased with time. Corticosterone

levels were lower in corticosterone treated rats than in vehicle treated rats at all time points, $F(1,10) = 10.71$, $p = 0.008$ (see Figure 12). For the rats treated in adolescence and tested several weeks later there was a significant main effect of time point, $F(2,24) = 6.42$, $p = 0.006$, such that corticosterone levels decreased with time. Although the interaction of treatment by time point missed statistical significance, $F(2,24) = 2.45$, $p = 0.11$, exploratory post hoc analyses were performed because the lack of statistical significance may have been because of low power. t-tests at each time point found that corticosterone levels in the corticosterone treated group were higher than those of the vehicle treated group only at the zero time point, $p = 0.04$ (see Figure 12).

A repeated measures ANOVA of corticosterone concentration for rats treated as adults indicated that there was a main effect of time point such that corticosterone concentration decreased with time after the FST, $F(2,8) = 8.12$, $p = 0.01$. Additionally, there was a main effect of timing of testing such that rats treated in adulthood and tested several weeks later had lower corticosterone levels than those tested immediately, $F(1,10) = 18.14$, $p = 0.02$.

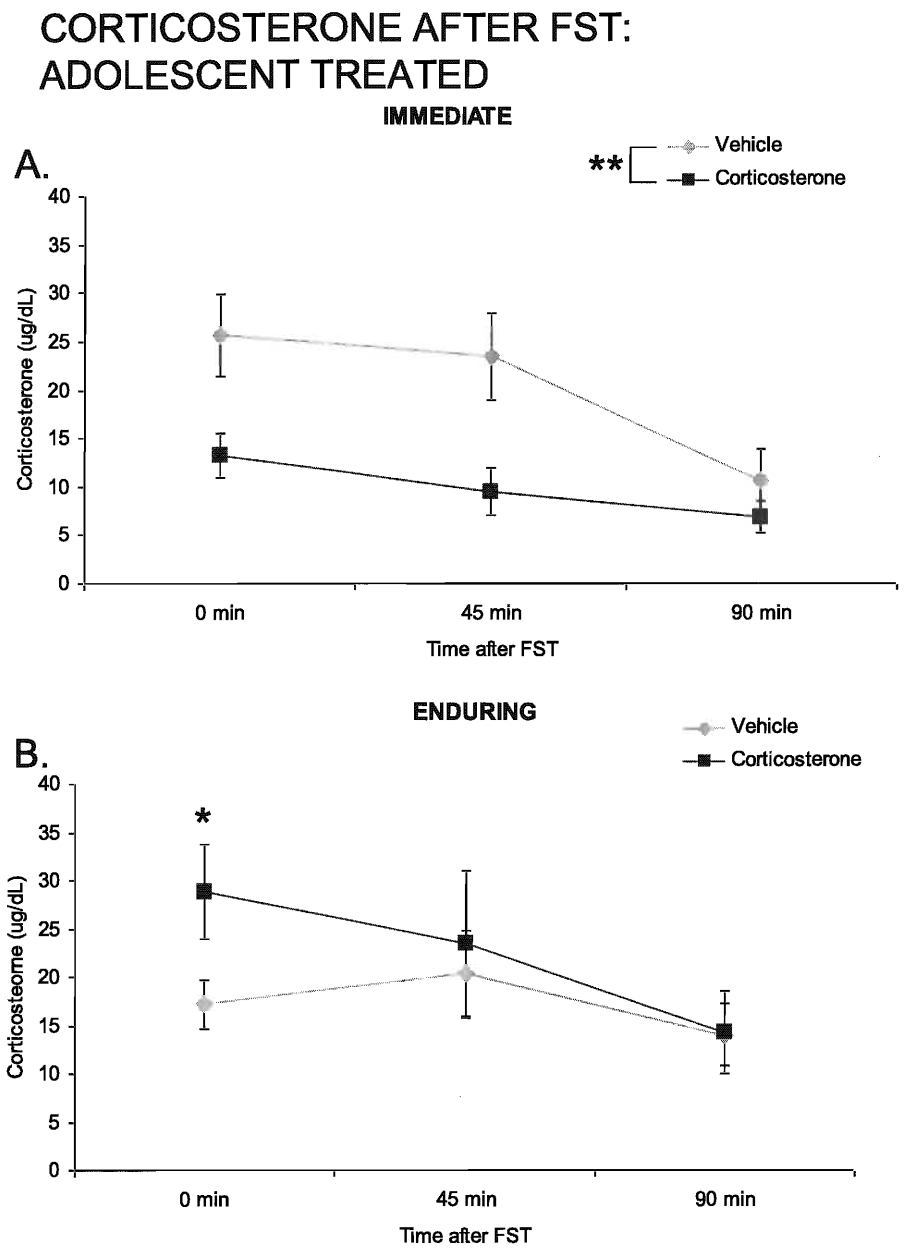


Figure 12. The means for the plasma concentrations of corticosterone measured after the FST for those adolescent rats tested A. soon after treatment and B. three weeks after treatment. Error bars represent the SEM. ** Indicates a main effect of treatment, $p = 0.008$. * Indicates a time point where corticosterone is significantly different from corresponding vehicle time point, $p = 0.04$.

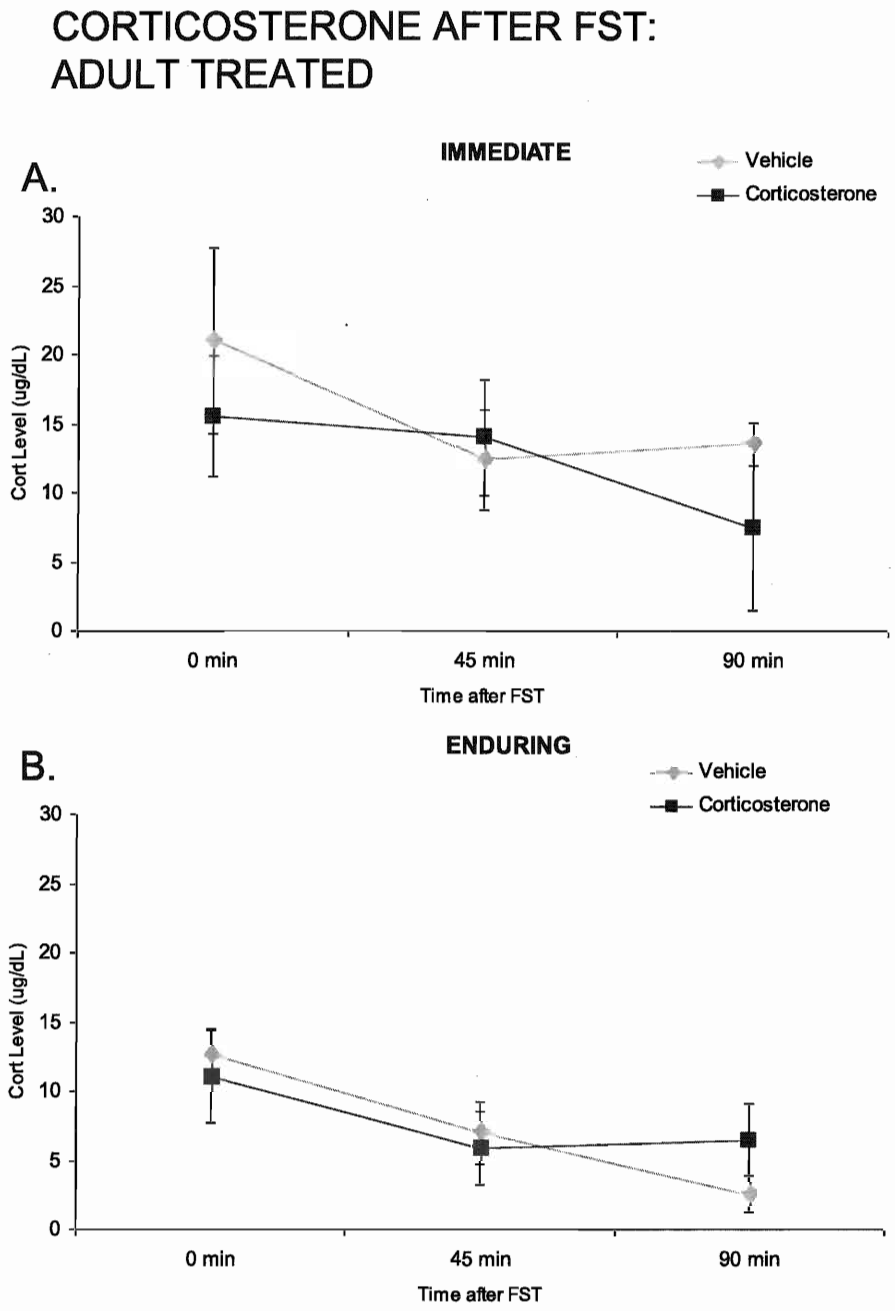


Figure 13. The means for the plasma concentrations of corticosterone measured after the FST for those adolescent rats tested A. soon after treatment and B. three weeks after treatment. Error bars represent the SEM.

Discussion for Experiment 1

The main findings for Experiment 1 were that rats treated with corticosterone injections in adolescence displayed no difference in anxiety-like or depressive behavior as measured by the EPM and FST when compared to rats injected with vehicle during adolescence regardless of when they were tested. Rats treated with corticosterone injections during adulthood and tested several weeks later displayed a mildly increased anxiety behavior pattern in the EPM, and increased climbing in the FST when compared to adult rats treated with vehicle. Rats treated with corticosterone injections in adolescence and tested immediately after treatment displayed a decreased HPA response to a stressor when compared to vehicle treated rats. Rats treated with corticosterone injections during adolescence and tested several weeks later showed an increased HPA response to a stressor when compared to vehicle treated rats. These results are discussed in greater detail below.

Weight

Rats treated with corticosterone had reduced weight gain compared to rats treated with vehicle that was evident as early as three days into the treatment. This is consistent with many previous reports that corticosterone administered in adulthood causes a decrease in body weight (Kalynchuk et al., 2004, Gregus et al., 2005, Johnson et al., 2006). Previous studies have also shown that chronic stress in adolescence (e.g., McCormick et al., 2007, Stone & Quartermain, 1997) or adulthood (eg. Matuszewich et al., 2007) causes reduced weight gain. Excess glucocorticoids, such as corticosterone, have been shown to induce insulin release, lipogenesis, gluconeogenesis, and protein catabolism, all of which individually could decrease body weight (reviewed in Dallman, 1984). Whereas stressed rats usually show recovery, such that within weeks they do not differ in weight within a few weeks after cessation of stress (McCormick et

al., 2004), the effects of corticosterone on weight were evident three weeks after treatment irrespective of the age at which they are treated. Thus, the dose of corticosterone used here surpasses the effects of most chronic stress paradigms on physiological responses.

In cage Behavior

Corticosterone treatment did not seem to affect behavior in the home cage after injections whether treated in adolescence or in adulthood. In contrast, stressors in adolescence do alter behavior. Acute restraint stress for 30 minutes at postnatal days 28, 35, 42, and 49 decreased play behaviors and increased time rats spent resting in groups in the home cage , when measured up to one hour after the stressor (Klein et al., 2010). This study also found that chronic restraint stress, 30 minutes every other day for 21 days decreased social investigation and increased time spent resting in groups when measured 24 hours after the last stressor (Klein et al., 2010). Other work has found that rats that experienced a social stressor in adolescence, one hour of isolation with a new cage partner after return to the home cage, daily for 16 days were less social than rats that had experienced a non-social stressor, isolation alone (McCormick et al., 2007). The discrepancy between these two studies may be explained by the different times at which behavior was monitored, the next day compared to immediate after stress, or the different durations of the stressor, 30 minutes compared to 60, or the administration period for the stressors, 21 days as opposed to 16. However, both studies do show chronic stressors experienced during adolescence have an effect on behavior in the home cage. It is possible that corticosterone did not have an effect on behavior in the home cage in the present experiment because the vehicle injections served as an acute stressor. Thus, both treatment groups may have been undergoing the effects of acute stress when they were monitored, thereby attenuating any treatment effect.

Anxiety-like and Depressive Behavior

Corticosterone treatment had no effect on any behavior in the EPM compared to vehicle treatment for the adolescent treated rats regardless of whether the rats were tested immediately or several weeks after treatment. There was no effect of corticosterone treatment during adolescence compared to vehicle treatment on either immobility or climbing behaviors in the FST. Adolescent rats treated with corticosterone and tested immediately spent more time swimming during the first five minutes of the FST than those treated with vehicle. However, because this effect was not accompanied by the expected increase in immobility after treatment with corticosterone, it should not be interpreted as decreased depressive behavior. Immobility is the behavior that represents the depressive state (Porsolt et al., 1977), and therefore without a difference in immobility, differences in other behaviors are not truly representative of an effect on depressive behavior. There was also a non-significant trend for the corticosterone treated rats to spend less time climbing during the first five minutes than those treated with vehicle. This may represent the corticosterone treated rats making less of a concerted effort to escape the test. Swimming around the border when the vehicle treated rats would have been climbing demonstrates a decreased effort to escape, which would be consistent with the literature that shows corticosterone treatment administered during adulthood increases depressive behavior in the FST as compared to rats treated with vehicle (Kalynchuk et al., 2004; Gregus et al., 2005; Johnson et al., 2006). Despite the marked effect of corticosterone treatment on weight, corticosterone did not produce the expected increase in depressive behavior in the FST or anxiety-like behavior in the EPM when administered either during adolescence or adulthood.

The majority of the literature shows that exposure to chronic stress of various durations during adulthood with a variety of stressors increases anxiety in the EPM (Vyas et al., 2002; 2004; Gamiero et al., 2006; Adamec et al., 2006; Pego et al., 2008; Bondi et al., 2008; Bowman

et al., 2009; Noschang et al., 2009). Additionally direct chronic administration of corticosterone, 25 mg/kg for 28 days, during adulthood has been shown to increase anxiety on the EPM (Pego et al., 2008). Both removing the adrenal glands and administering drugs that prevent the production of corticosterone abolish the increased anxiety observed in rats exposed to chronic stress (Calvo et al., 2001). Treating rats with a GR antagonist also prevents the increased anxiety induced by chronic stress (Korte et al., 1995). Taken together these studies show that stress increases anxiety because of the exposure to corticosterone.

The reason corticosterone was not observed to increase anxiety or depressive behavior compared to vehicle treated rats when administered during adolescence may be that the vehicle injections were stressful enough to also produce an increase in anxiety. This would mean that the injections were stressful enough that the corticosterone had no effect beyond what the injections themselves had already caused. One problem for this last possibility, though, is that I found no difference between the treatment groups when administered in adulthood, whereas there are several reports of differences between corticosterone-injected and vehicle-treated rats in depressive behavior in the FST (Kalynchuk et al., 2004; Gregus et al., 2005; Johnson et al., 2006; Lee et al., 2009). However, a cross comparison of studies demonstrates that duration of the administration of corticosterone is an important factor in demonstrating increased depressive behavior. Those studies with durations of corticosterone administration that are longer than that used here, 16 days, do show corticosterone treatment increased depressive behavior in the FST, but studies using a duration of administration shorter than that used here do not show (reviewed in Sterner & Kalynchuk, 2010). Thus, it may be that 16 days of corticosterone treatment is insufficient number of days for corticosterone treatments to be effective at either age. Thus, no support was found for that adolescents would show greater lasting effects of corticosterone

treatment on anxiety-like and depressive behavior compared to adults. It is also possible that the reason for the lack of treatment effects is for different reasons at each age: effect of stress of injection masking the effect of corticosterone compared to vehicle in adolescents, whereas in adults, the length of treatment was insufficient. A second experiment was designed to remove the possibility of injection stress (described later).

The design of the experiment allowed me to also investigate age differences in behavior on the FST and on the EPM by comparing the behavior of rats tested as adolescents to those tested as adults within the adolescent treatment groups. Adolescent rats spent less time on the open arm, had a higher latency to reach the open arm, a lower percentage of entries into the open arm, performed fewer head dips, and fewer rears than did adult rats (postnatal day 70), all indications of greater anxiety-like behavior in adolescence than in adulthood (Wall & Messier, 2001; Doremus et al., 2006). Additionally, adolescent rats were less active (entries into closed arms) and spent less time assessing risk (time in center) than those tested several weeks after treatment. Adolescent rats also displayed decreased levels of depressive behavior on the FST in that they had a higher latency to immobility, spent less time immobile and spent more time swimming and climbing than did adults. Other studies have shown adolescents, postnatal day 45 and 48, as acting less anxious than adults, postnatal day 60 and 61, in the EPM (Imhof et al., 1993, Macri et al., 2002). Another study has shown young adolescent mice, postnatal day 21 to have decreased depressive behavior in the FST as compared to mice in mid-adolescence, postnatal day 42, and adults, postnatal day 56, which were not different from one another (Hefner & Holmes, 2007). Therefore the results reported here are not consistent with other literature, but it is possible that the age difference observed here represents recovery from a stressor, injections. Adolescents have been shown to be more susceptible to the effects of

stressors than adults (reviewed in McCormick et al., 2010), and therefore it is possible that the adolescents of either treatment group were receiving a chronic stressor. The behavior of the adolescents tested several weeks after treatment was similar to that of the adults tested immediately, therefore those groups are most likely reflecting typical adult behavior. It is impossible to determine from this experiment whether the age difference observed here reflects an age difference or recovery from the stress of injection. Therefore a second experiment was designed with the purpose of observing the effects of corticosterone without the confounding stress of injection, which will be discussed in the second portion of this report.

The adult rats treated with corticosterone produced unusual behavior patterns on the EPM and the FST as compared to adults treated with vehicle. However, these rats were severely underweight, and consequently they were suffering from health issues. It is entirely unclear whether their behavior could be attributed to the corticosterone treatment or the health issues they experienced as a consequence of the corticosterone treatment. Therefore a second experiment employed a less severe method of administering corticosterone, which will be discussed in the second portion of this report.

The variables measured on the EPM were correlated with one another in a manner that was expected based on factor analyses of behavioral measures the EPM (reviewed in Wall & Messier, 2001), which provides some indication that the analysis of behavior in the EPM was performed appropriately.

HPA Response to a Stressor for Adolescent Treated Rats

Despite the lack of differences in behavior between corticosterone and vehicle treated rats, the groups differed in corticosterone release after the FST. When tested soon after treatment in adolescence, corticosterone concentrations were lower in corticosterone-treated rats than in

vehicle injected controls. This is consistent with the finding that chronic corticosterone treatment in adults reduced corticosterone response to forced swim (Johnson et al., 2006). The effect observed here is likely the result of severe inhibition of the adrenal gland. Chronic administration of high levels of corticosterone would increase activation of the negative feedback system within the HPA, which would shut down corticosterone production in the adrenal gland. The immediate testing most likely did not allow sufficient time for the adrenal gland to recover from such high levels of inhibition, and therefore the adrenal gland would have depleted its stores of corticosterone. Therefore the dampened corticosterone response to a stressor observed in adolescent treated rats tested soon after treatment is likely the result of chronic corticosterone treatment shutting down corticosterone production in the adrenal gland.

When tested several weeks after treatment, corticosterone-treated rats displayed an increased release of corticosterone in response to the FST compared to vehicle injected controls. Thus, exposure to high levels of corticosterone in adolescence produced an enduring change in the HPA axis, such that the release of corticosterone in response to a stressful event was increased. The reports of enduring effects of chronic stressors on later HPA function are mixed. Consistent with my results, Isgor et al. (2004) found a prolonged corticosterone response to a novel stressor in adulthood after repeated unpredictable physical stress during adolescence. Several studies have found unaltered HPA response in adult males after experiencing a stressor during adolescence (Maslova, et al., 2002, Mathews et al., 2008, McCormick et al., 2005, Overmier & Murison, 1991, Toledo-Rodriguez & Sandi, 2007, Wright et al., 2008). Other studies have found changes in basal levels of corticosterone in adult males that had experienced chronic stress during adolescence (Toth et al., 2008, Sterlemann et al., 2008, Schmidt et al., 2007, Uys et al., 2006a, Uys et al., 2006b, Shaffery et al., 2006, Lepsch et al., 2005). Our findings indicate that

high levels of corticosterone present during adolescence alter the development of the HPA axis such that it becomes more reactive to stressors experienced in adulthood. This effect may require repeated exposures to consistently high levels of corticosterone. Thus, even though injection alone is stressful, it does not mimic the effect of corticosterone injection on stress responses. Further these results show that the lack of behavioral results may be substantive rather than representative of an insufficient dose because effects were observed on the HPA.

One possible mechanism for the heightened HPA response in rats treated with corticosterone during adolescence and tested several weeks after treatment is neuronal remodeling in the hippocampus. Several studies have shown that chronic stress (Galea et al., 1997; Vyas et al., 2002) and corticosterone administration (Woolley et al., 1990; Watanabe et al., 1992; Magarinos et al., 1998; Cerqueira et al., 2005; 2007) can cause dendritic atrophy in the hippocampus of adults. The hippocampus is an important site of negative feedback for the HPA axis, and therefore damage to the hippocampus may decrease the negative feedback produced in response to high levels of circulation corticosterone (reviewed in de Kloet et al., 1998). This is a suggested mechanism for the hypercortisolism observed in patients with depression (reviewed in de Kloet et al., 1998). In adults dendritic atrophy in the hippocampus resulting from stress is reversible (Vyas et al., 2004). However, the hippocampus is a site of ongoing development during the adolescent (reviewed in Lupien et al., 2009), and therefore dendritic atrophy in the hippocampus that occurs during adolescence may be longer lasting than that observed during adulthood. It is possible that remodeling in the hippocampus as a result of corticosterone exposure during adolescence is the mechanism behind the increased HPA response observed in the rats treated with corticosterone during adolescence and tested several weeks after treatment as compared to those treated with vehicle injections.

The same argument could be made for the prefrontal cortex as the locus of the mechanism for increased HPA response observed in corticosterone treated adolescents tested several weeks after treatment as compared to the corresponding vehicle treated control group. The prefrontal cortex undergoes dendritic reorganization after exposure to chronic stress during adulthood (Wellman, 2001; Seib & Wellman, 2003). The prefrontal cortex is also a site of negative feedback for the HPA axis (reviewed in de Kloet et al., 1998), and the prefrontal cortex undergoes development during adolescence (reviewed in Lupien et al., 2009). Therefore it is just as possible that corticosterone exposure during adolescence induces enduring alterations in dendritic morphology of the prefrontal cortex, which is in turn responsible for the decreased HPA control resulting in the increased HPA response observed in those adolescents treated with corticosterone and tested several weeks after treatment as compared to the corresponding vehicle treated control group. The lasting effects of corticosterone may be entirely peripheral to the nervous system, since there was no marked effect of corticosterone treatment on behavior but extreme effects on weight and corticosterone release. It is possible that the differences in corticosterone release observed are based entirely at the level of the adrenal gland.

HPA Response to a Stressor for Adult Treated Rats

There was some support for the hypothesis that the effects of corticosterone treatment on corticosterone release would be greater when administered in adolescence than when administered in adulthood. There was no effect of corticosterone treatment during adulthood on corticosterone response to a stressor regardless of whether the rats were tested for immediate or enduring effects. For those adult treated rats tested immediately, the inability to replicate decreased corticosterone response observed in the adolescent treated rats in the present study and in other studies of adult rats (Johnson et al., 2006) is likely a result of the small sample size. The

dramatic weight loss over the course of the experiment in adult rats made the collection of blood samples more difficult than anticipated. Among those adults tested several weeks after treatment there is scant difference in corticosterone response to the FST. Although it is possible that an effect of corticosterone treatment would become apparent with a larger sample size, it is more likely that there is no enduring effect of corticosterone treatment on the adult HPA.

Conclusions

The results from this experiment indicate that corticosterone administration during adolescence can have enduring effects on the HPA axis and on weight. However, effects of corticosterone administration on anxiety-like and depressive behavior were not observed. One possible explanation for the lack of behavioral effects is that the corticosterone treated group was being compared to a group of rats that had undergone a chronic mild stressor, the vehicle injections. Further investigation is required to determine if corticosterone administration can affect anxiety-like and depressive behavior when compared to unstressed controls, and if adolescents are more sensitive to corticosterone treatment than are adults.

Introduction: Experiment 2

As discussed above, one possible explanation for the lack of clear effects of corticosterone on the EPM and FST may be that the vehicle injections represent a stressor, which would have similar effects to corticosterone administration. Therefore both groups may be experiencing the same effects on behavior. The purpose of the second experiment was to circumvent this issue by administering corticosterone in the drinking water. This way rats would receive corticosterone without any additional stressors, and the control group would be without a potentially confounding stressor.

Administering corticosterone in the drinking water at a concentration of 400 μg/mL dissolved with 2.4% ethanol for 21 days has been shown to elevate plasma levels of corticosterone in adult rats such that concentrations consistently remain at levels similar to that experienced at the diurnal peak (Magarinos et al., 1998). This study also demonstrated that corticosterone administration in drinking water reduced weight compared to ethanol treated controls (Magarinos et al., 1998). However, when measured two weeks after treatment, corticosterone administered in the drinking water, 50 μg/mL, had no effect on basal levels of corticosterone in adult rats (Gourley &Taylor, 2009). Corticosterone administration in drinking water at a concentration of 35 μg/mL for 28 days, has also been shown to dampen the corticosterone response to a novel stressor when tested soon after treatment (David et al., 2009). These studies demonstrate that extended exposure to corticosterone in drinking water can affect the HPA axis in adults, but the effects are not enduring.

Corticosterone administration in the drinking water has also been demonstrated to alter anxiety-like and depressive behavior in rodents. Administering 50 μg/mL of corticosterone to

rats or 25 μg/mL to mice dissolved in the drinking water for 14 days with a 6 day weaning period, whereby the dose was decreased incrementally decreased, increased depressive behavior in the FST and decreased sucrose consumption, a measure of anhedonia in the rodent (Gourley et al., 2008; Gourley & Taylor, 2009). However, mice given 35 μg/mL corticosterone in their drinking water for four weeks demonstrated no effect on their behavior in the FST compared to vehicle treated controls, but corticosterone treatment did increase time spent in the center of the open field test, which is indicative of higher levels of anxiety (David et al., 2009). However, when rats were tested two weeks after treatment, corticosterone treatment for 14 days with six days of the aforementioned weaning procedure, did not affect anxiety-like behavior on the EPM (Gourley & Taylor, 2009). These studies do not provide a clear view of the effects of corticosterone on behavior, possibly because of the different doses and durations of administrated drug. It is also possible that the weaning period used initially by Gourley et al. (2008) alters the effects of corticosterone on behavior in a different manner than corticosterone that is given at a consistent dose. Further study would be required to determine how corticosterone administered in drinking water can affect these behaviors.

The present study will demonstrate whether corticosterone administered in the drinking water during either adulthood or adolescence can produce an effect on depressive behavior and HPA response to a stressor and if those effects will be enduring. It is predicted that depressive behavior will increase in rats treated with corticosterone, and that increased depressive behavior would be longer lasting in adolescent treated rats. It is predicted that for rats of either age tested soon after treatment, corticosterone will dampen the HPA response to a stressor, as it did in Experiment 1. It is also predicted that for rats tested several weeks after treatment and treated during adolescence, the HPA response to a stressor will be increased, as it was in Experiment 1,

but there will be no enduring effect of corticosterone treatment in adult rats tested several weeks after treatment.

Methods: Experiment 2

Animals

74 male Long-Evans rats, obtained from Charles River, St. Constant, Quebec, arrived in the colony, 36 of them at postnatal day 22, 16 at postnatal day 60 and 20 at day postnatal 67. They were housed in pairs in polycarbonate cages and were provided with one plastic tube in each cage for enrichment. Rats were identified by tail coloring with a felt tip marker. The rats were kept on a 12 hour light, 12 hour dark, light cycle, and were given unlimited access to rat chow and water at all times. Rats were given at least four days to adjust to these housing conditions before the beginning of the experimental procedures. All experimental procedures were consistent with National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), and Canadian Council on Animal Care guidelines and were approved by the Brock University Institutional Animal Care and Use Committee.

Drinking Water Treatment

At postnatal day 30 or 70 the drinking water was replaced with either a solution of 400 µg / mL corticosterone (purchased from Steraloids, Newport, RI) and 2.5 % ethanol (n = 32, half started on day 30 and half on day 70), which is necessary to get the corticosterone to dissolve. To ensure that any effects observed were the result of corticosterone treatment and not ethanol exposure, a second group was given 2.5% ethanol (n = 20, half started on day 30 and half on day 70), and the third group was given plain water (n = 20, half started on day 30 and half on day 70). Each solution was colored with a different food coloring so that they would be easily distinguished from one another. After 16 days, on postnatal day 46 or 86, regular drinking water (tap water) was provided and treatment was removed. During treatment, the mass of water

consumed was measured every two days. Rats were weighed before and after the 16 days of treatment, but were not handled during the course of treatment, except for cage cleaning, which occurs three times a week. Using weight at the starting and ending of treatment, the dose of ethanol and of corticosterone consumed by the pair of rats in a cage over a two day period was calculated. To calculate the doses of ethanol and corticosterone, the density of each solution was determined by weighing one mL of each solution, because consumption was measured in mass. Using the beginning and ending weights, the approximate weight at the eighth day of treatment was extrapolated, and the ethanol and corticosterone doses for the seventh and eighth day were calculated based on this weight.

Forced Swim Test

All rats in this experiment were tested on the FST, either soon after treatment (postnatal day 47 or 87, depending on age at treatment) or long after treatment (postnatal day 71 or 111, depending on age at treatment) using the same procedure for the FST as described in experiment one.

Plasma Corticosterone Determination

To determine the amount of corticosterone released after the FST, blood was collected by tail nick from each of the rats immediately, 45 minutes and 90 minutes after removal from the test. On the next day, a blood sample was obtained to provide a baseline level of corticosterone. No baseline was obtained before the FST in order that behavior in the FST would not be affected by the blood sampling. All blood samples were centrifuged at 3000 rpm and 4 °C for twenty minutes, and then the plasma was collected. Plasma samples were stored at -20 °C until they were measured. Corticosterone concentrations were determined using an enzyme-linked immunosorbent assay kit (purchased from Neogen, Lansing MI). Corticosterone was extracted

from the samples by dissolving it in ethyl ether and then removing the organic layer containing the ether and all steroids from the original sample. The ether was evaporated off and the steroids were reconstituted in a buffer provided in the kit. The assay was run entirely as specified in the instructions for the kit; except that the reconstituted samples were diluted two fold what is instructed in the kit, in order for stress levels of corticosterone to be readable within the standard curve. The minimum detection level for the assay is 1 μg per tube. The antiserum cross-reacts with deoxycorticosterone (38%), and only slightly with cortisol (1.1%), testosterone (0.12%), and estradiol (< 0.01%). The intra- and inter-assay reliability are both less than 10%. The samples for experiment 2 were run at two different times, once included all rats tested in the FST two days after removal of treated water, and another run consisted of all the rats tested 25 days after the removal of treated water.

Statistical Analysis

As in Experiment 1, analyses were performed separately on adolescent-treated and adults-treated. Weight differences were analyzed using repeated measures ANOVAs with the between subject factor of treatment. Behavior during the FST was analyzed using a repeated measures ANOVA for each variable considered with treatment and timing of testing as the between subjects factors, the results of which can be found in Appendix A. This was followed up with a repeated measures ANOVA for each variable considered at each timing of testing group with treatment as the between subject factor. The plasma levels of corticosterone were analyzed using a repeated measures ANOVA with timing of testing and treatment as the between subjects factors. This was followed up where appropriate with a repeated measures ANOVA for each timing of testing group with treatment as the between subject factor. Basal levels of corticosterone were analyzed with a separate ANOVA for each timing of testing group using

treatment as the between subject factor. Fisher's protected least square differences were used for post hoc analyses where appropriate. An alpha level of $p < 0.05$ was considered significant.

Table 4.
Sample sizes of groups for each measure in Experiment 2.

	Immediate			Enduring		
	CORT	ETHANOL	WATER	CORT	ETHANOL	WATER
Adolescents						
Weight	8	6	6	8	4	4
Intake[1]	4	3	3	4	2	2
FST	8	6	6	8	4	4
Cort [2]	8	6	5	8	4	4
Adults						
Weight	8	6	6	8	4	4
Intake[1]	4	3	3	4	2	2
FST	8	6	6	8	4	4
Cort [2]	6	5	3	8	4	4

1. N indicates number of bottles, each of which is shared between two rats.
2. Corticosterone measured after the FST data were only analyzed for those rats from which we were able to collect a usable sample at all three time points.

Results: Experiment 2

In all data analyses adolescents and adults are analyzed separately, except for the doses of ethanol and corticosterone. In these cases it is important to note whether the ages were receiving different doses of either ethanol or corticosterone. Additional statistical analyses can be found in Appendix A.

Weight

For rats treated in adolescence, a repeated measures ANOVA of weight measured on the first and last days of treatment for the rats found an interaction of treatment and time point, $F(2,33) = 12.91$, $p < 0.001$. This was followed up with a separate ANOVA of weight for each day weight was measured. There was no significant difference between treatment groups on the first day of treatment. On the last day of treatment there was a significant effect of treatment, $F(2,33) = 7.41$, $p = 0.002$, and the post hoc analyses showed that corticosterone treated rats weighed less than ethanol treated rats, $p = 0.035$, and corticosterone treated rats weighed less than rats given plain water, $p = 0.001$, but water and ethanol groups were not significantly different from one another (see Figure 14).

WEIGHT

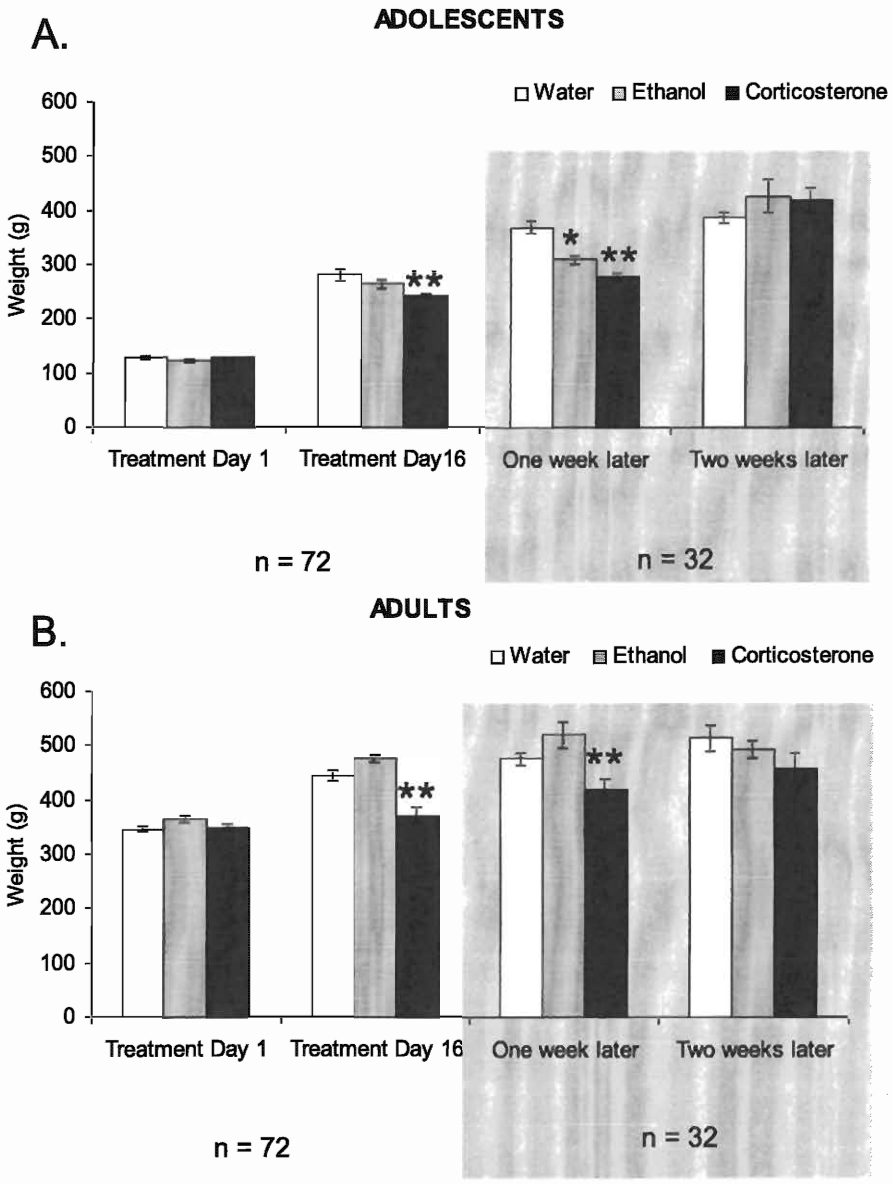


Figure 14. The means for the body weights of A. adolescents and B. adults during treatment (unshaded) and for those rats tested three weeks later (shaded). Error bars represent the SEM. * Indicated significantly different from the water group, p = 0.01, and ** indicates significantly different from both corresponding ethanol and water treatment groups, p = 0.05 or less.

For the adult treated rats, a repeated measures ANOVA of weight measured on the first and last days of treatment found a significant interaction between treatment and time point, $F(2,33) = 18.92$, $p < 0.001$. This was followed up with a separate ANOVA of weight for each day it was measured. On the first day of treatment there was no significant difference between treatment groups $F(2,33) = 2.60$, $p = 0.08$, although rats given ethanol tended to be heavier. On the last day of treatment there was a significant effect of treatment, $F(2,33) = 20.89$, $p < 0.001$. Corticosterone treated rats weighed less than ethanol treated rats, $p < 0.001$, and less than rats with plain water, $p < 0.001$. Ethanol and water groups were not significantly different from one another (see Figure 14).

A repeated measures ANOVA of weight measured after treatment for the rats treated in adolescence and tested several weeks later indicated that there was a significant interaction between week the weight was measured and treatment, $F(2,13) = 5.73$, $p = 0.02$. This was followed up with individual ANOVAs of weight for each week. At the first week after treatment there was a significant effect of treatment, $F(2,13) = 26.05$, $p < 0.001$. The post hoc analyses indicated that corticosterone treated rats weighed less than ethanol treated rats, $p = 0.03$, that corticosterone treated rats weighed less than rats with plain water, $p < 0.001$, and that ethanol treated rats weighed less than those with plain water, $p = 0.01$. By the second week after treatment there was no effect of treatment on weight (see Figure 14).

For rats treated as adults, the main effect of treatment of weight in the weeks after treatment was significant, $F(2,13) = 5.42$, $p = 0.02$. Corticosterone treated rats weighed less than ethanol treated rats, $p = 0.01$, corticosterone treated rats weighed less than those with plain water, $p = 0.03$, but ethanol and water groups did not differ.

Treated Water Intake

For the adolescent treated rats, a repeated measures ANOVA of two days worth of treated water intake on the second, eighth, and sixteenth days of treatment indicated a significant main effect of time point $F(2,30) = 15.24$, $p < 0.001$, with intake increasing over the course of treatment. There was an effect of treatment, $F(2,15) = 4.08$, $p = 0.04$. Corticosterone treated rats drank less than the plain water group, $p = 0.02$, ethanol treated rats drank less than the water group, $p=0.05$, but the corticosterone and ethanol groups did not differ.

For adult-treated rats, there was a main effect of time point such that rats drank more over time in all groups, $F(2,30) = 6.43$, $p = 0.005$, but there was no effect of treatment, $F(2,15) = 1.80$, $p = 0.20$ (see Figure 15).

INTAKE

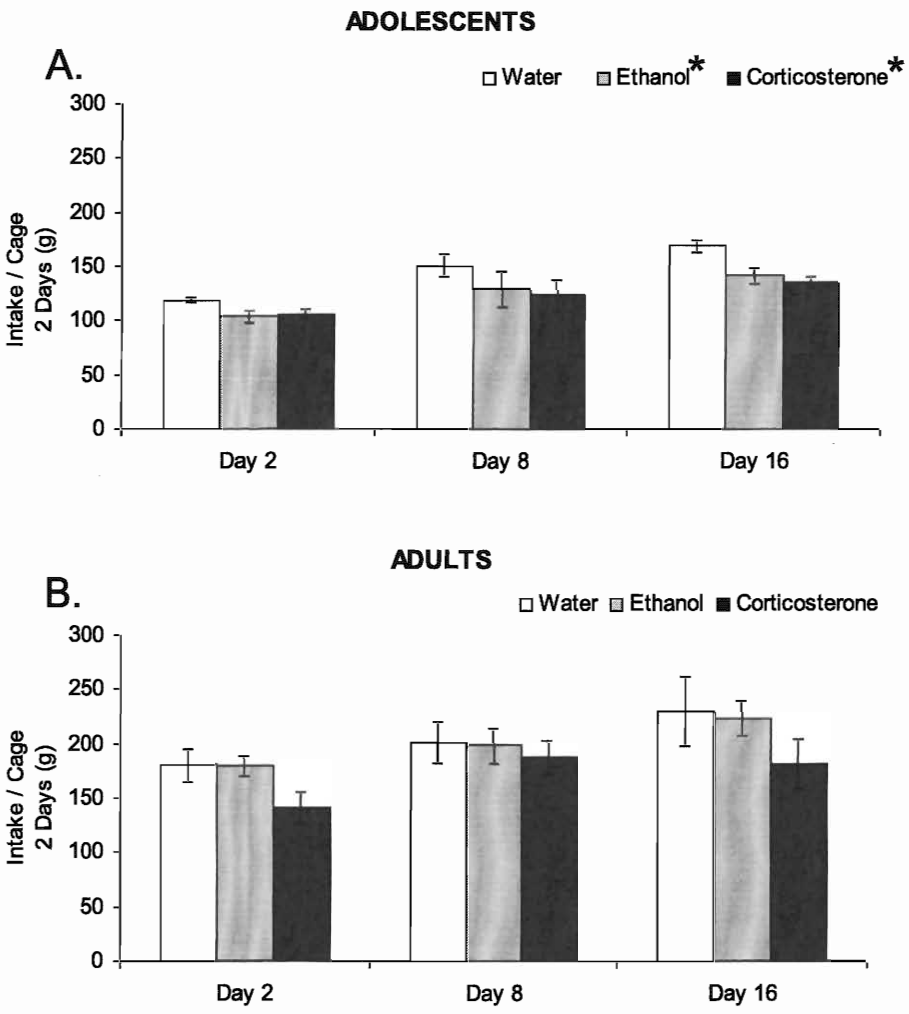


Figure 15. The means for the amount of treated water drank per cage, i.e. by two rats, in two days for A. adolescent treated rats and B. adult treated rats. Error bars represent the SEM. * Indicates groups significantly different from water, p = 0.05 or less.

Ethanol Dose

A repeated measures ANOVA of ethanol dose on the second, eighth, and sixteenth days of treatment showed that there was a significant interaction between day of treatment and age group, $F(2,36) = 9.12$, $p = 0.001$. Follow up repeated measures ANOVAs of ethanol dose for each age group showed that for the adolescent treated rats ethanol dose decreased with time, $F(2,24) = 16.69$, $p < 0.001$, but not for the adult treated rats, $F(2,24) = 1.04$, $p = 0.37$ (see Figure 16). Follow up independent samples t-test indicate that the adolescent treated rats ingested a larger dose of ethanol on the first two days of treatment, $p < 0.001$, and a near significant trend for the seventh and eighth days of treatment, $p = 0.06$, but not by the last two days of treatment, $p = 0.20$.

Corticosterone Dose

A repeated measures ANOVA on corticosterone dose found an interaction between day of treatment and age, $F(2,28) = 6.05$, $p = 0.007$. For the first two days of intake the adolescents consumed a higher dose of corticosterone than the adults $p < 0.001$, but not for the seventh and eighth days, $p = 0.13$ or for the last two days of intake, $p = 0.41$ (see Figure 16).

DOSAGE

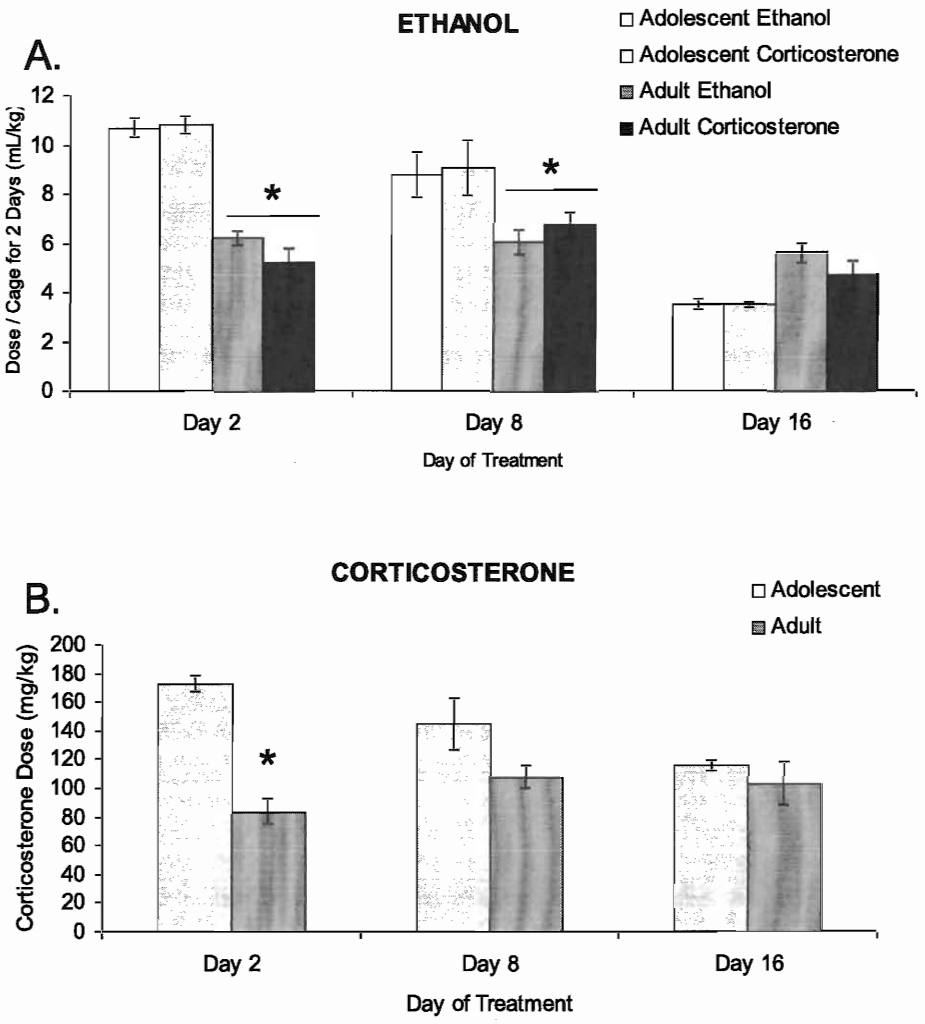


Figure 16. The means for the average dose ingested by one cage, i.e. by two rats, in two days measured at three time points across treatment for rats treated with A. Ethanol, * indicates a significant difference between age groups at that time point, $p = 0.01$ or less. or B. Corticosterone, * indicates that the adults ingested a significantly lower dose, $p < 0.001$. Error bars indicate the SEM.

Forced Swim Test

Latency to Immobility: An ANOVA of latency to remain immobile for a minimum of five seconds in the adolescent treated rats indicated that there was a main effect of time of testing such that adolescent treated rats tested immediately took more time than those tested several weeks later to become immobile, $F(1,30) = 22.90$, $p < 0.001$, but the same was not true for the adult treated rats, $F(1,30) = 0.97$, $p = 0.33$ (see Figure 17).

Duration of Immobility: For adolescent treated rats there was a significant effect of time point, $F(3,90) = 76.31$, $p < 0.001$, such that as the test progressed rats spent more time immobile. There was a significant effect of time of testing, $F(1,30) = 28.35$, $p < 0.001$, such that rats treated in adolescence and tested several weeks later spent more time immobile than those tested immediately. There was no effect of treatment, $F(2,30) = 0.12$, $p = 0.89$, and no interaction between time of testing and treatment, $F(2,30) = 0.17$, $p = 0.84$ (see Figures 18 and 19).

For adult treated rats there was a significant effect of time point, $F(3,90) = 88.61$, $p < 0.001$, such that as the test progressed rats spent more time immobile. There was no effect of time of testing, $F(1,30) = 0.71$, $p = 0.41$, no effect of treatment, $F(2,30) = 1.00$, $p = 0.38$, and no interaction between time of testing and treatment, $F(2,30) = 0.48$, $p = 0.62$ (see Figures 20 and 21).

LATENCY TO IMMOBILITY

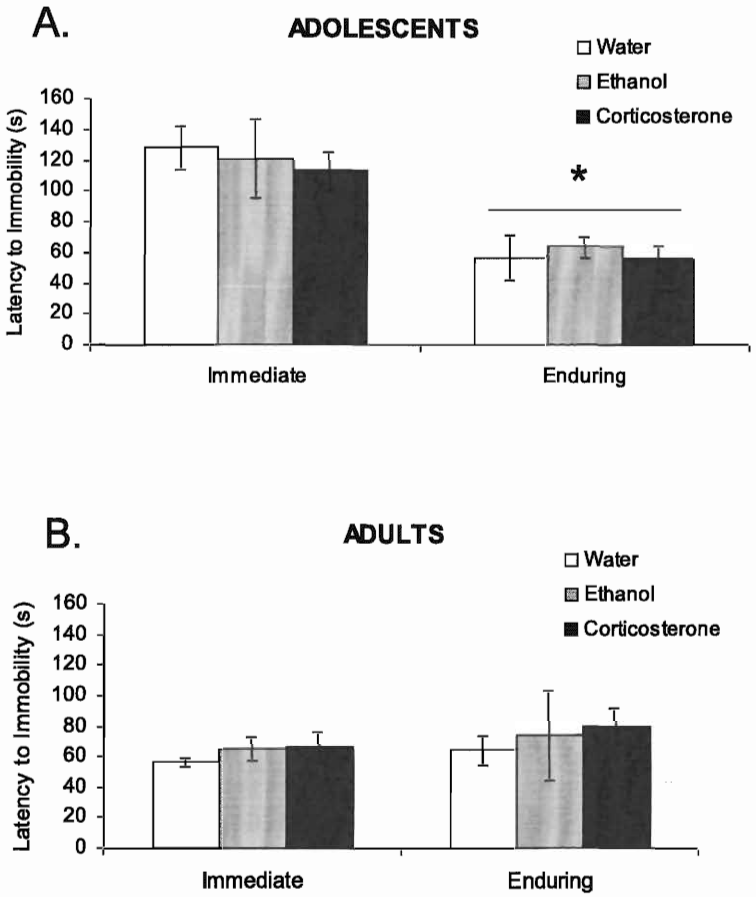


Figure 17. The means for the amount of time taken before spending a minimum of five seconds immobile in the FST for A. the adolescent treated rats and B. the adult treated rats. Error bars represent the SEM * Indicates a main effect of time of testing, $p<0.000$.

Duration of Swimming: For the adolescent treated rats those rats that were tested several weeks after treatment swam less than those rats that were tested immediately after treatment, $F(1,30) = 22.64$, $p < 0.001$, and there was a main effect of time point such that adolescent treated rats swam less as the test progressed, $F(3,90) = 3.72$, $p = 0.014$. There was no effect of treatment, $F(2,30) = 0.01$, $p = 0.99$, and there was no interaction between time of testing and treatment, $F(2,30) = 0.07$, $p = 0.94$ (see Figures 18 and 19).

For the adults there was no effect of time point, $F(3,90) = 1.33$, $p = 0.27$, no effect of time of testing, $F(1,30) = 2.39$, $p = 0.13$, no effect of treatment, $F(2,30) = 1.95$, $p = 0.16$, and no interaction between time of testing and treatment, $F(2,30) = 1.45$, $p = 0.25$ (see Figures 20 and 21).

ADOLESCENT TREATED AND TESTED IMMEDIATELY

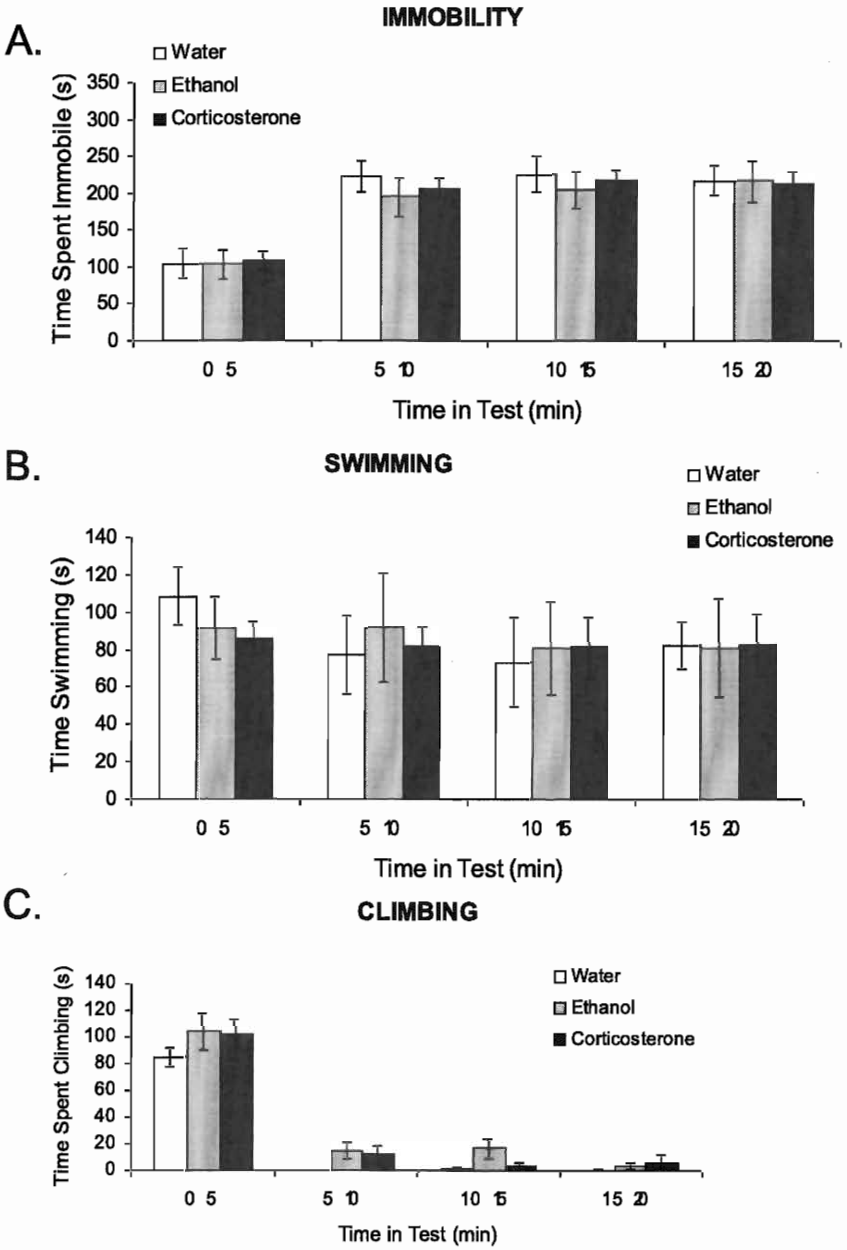


Figure 18. The means for behaviors performed by adolescents tested immediately during the FST, A. immobility, B. swimming, and C. climbing. Error bars represent the SEM.

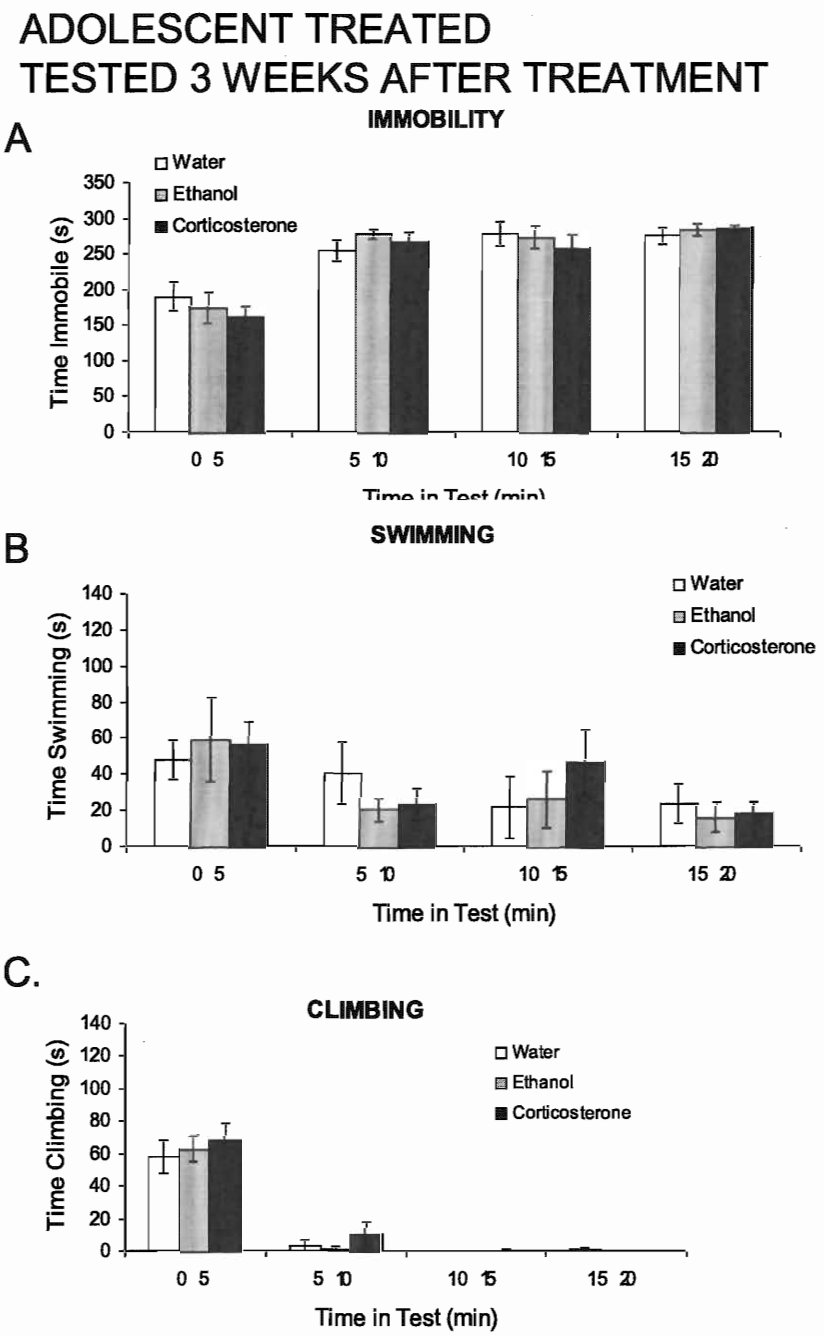


Figure 19. The means for behaviors performed by adolescents tested three weeks following treatment during the FST, A. immobility, B. swimming, and C. climbing. Error bars represent the SEM.

Duration of Climbing: Because there was an interaction between time point and time of testing at both time of treatment ages (results reported in Appendix A), the time of testing groups were analyzed separately for each age group.

For rats treated in adolescence and tested immediately, rats climbed less as the test progressed, $F(3,51) = 150.47$, $p < 0.001$, but there was no effect of treatment on time spent climbing, $F(2,17) = 2.10$, $p = 0.15$ (see Figure 22). For rats treated in adolescence and tested several weeks, rats later climbed less as the test progressed, $F(3,39) = 64.99$, $p < 0.001$, but there was no effect of treatment on climbing, $F(2,13) = 0.61$, $p = 0.56$ (see Figure 19).

For adult treated rats tested immediately, rats climbed less as the test progressed, $F(3,51) = 145.70$, $p < 0.001$, but there was no effect of treatment, $F(2,17) = 1.36$, $p = 0.28$ (see Figure 24). For rats treated as adults and tested several weeks later, rats climbed less as the test progressed, $F(3,39) = 57.88$, $p < 0.001$, but there was no effect of treatment, $F(2,13) = 0.07$, $p = 0.94$ (see Figure 21).

ADULT TREATED AND IMMEDIATELY TESTED

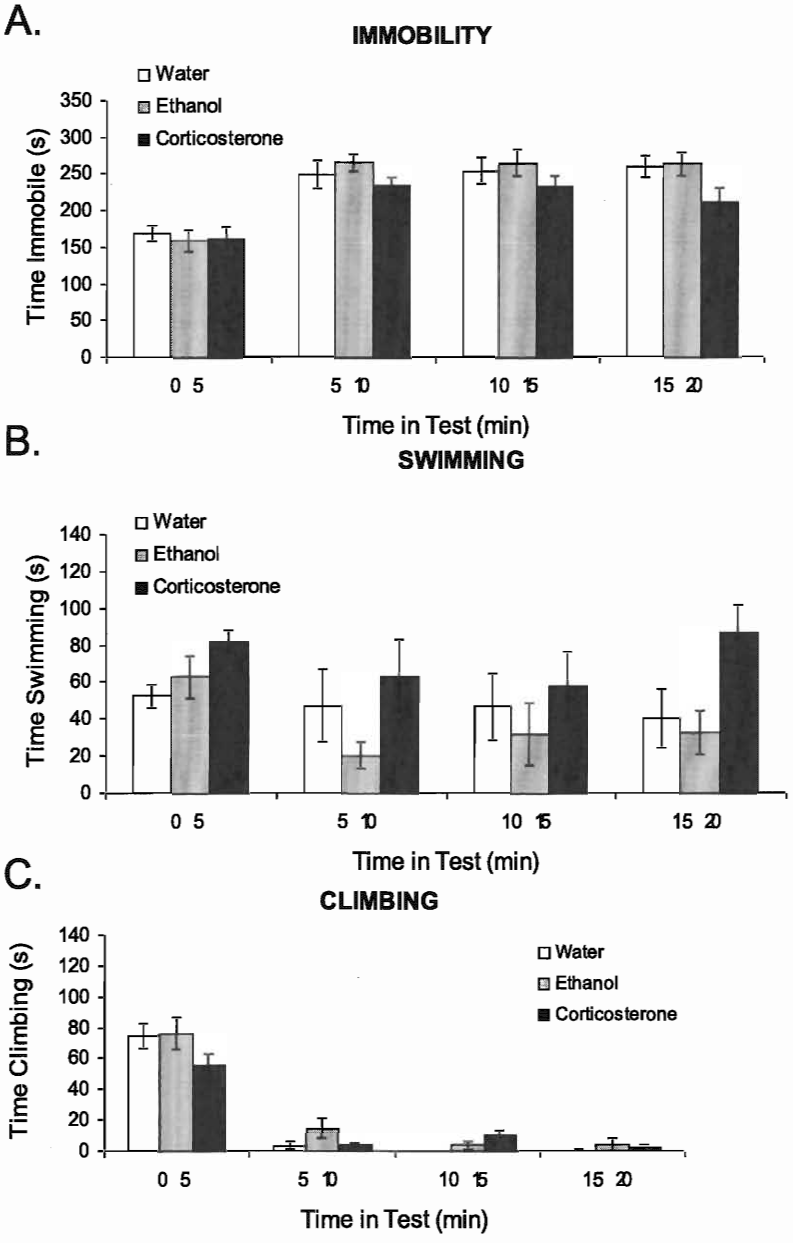


Figure 20. The means for behaviors performed by adults tested immediately following treatment during the FST, A. immobility, B. swimming, and C. climbing. Error bars represent the SEM.

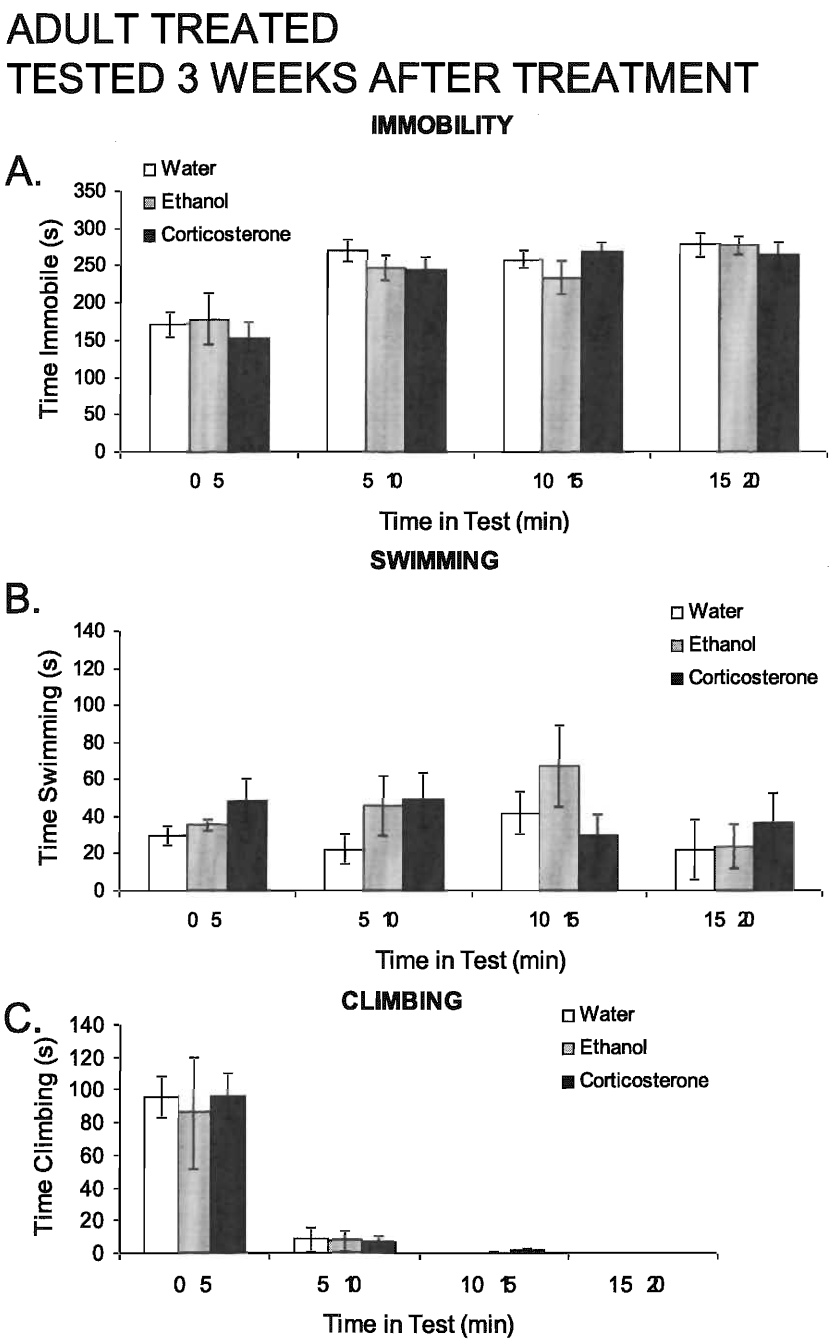


Figure 21. The means for behaviors performed by adults tested three weeks after treatment in the FST, A. immobility, B. swimming, and C. climbing. Error bars represent the SEM.

Corticosterone levels after the Forced Swim Test

For adolescent treated rats, there was a significant time point by treatment by time of testing interaction on corticosterone levels, $F(4,60) = 3.54$, $p = 0.01$. This was followed up with repeated measures ANOVAs of corticosterone concentrations at the three measured time points after the FST for each time of testing group. For the rats treated in adolescence and tested immediately the interaction between time point and treatment was significant, $F(4,34) = 5.82$, $p = 0.001$. This was followed up with univariate ANOVAs of corticosterone concentration at each time point. Post hoc analyses indicated that at the zero time point, the corticosterone group had lower corticosterone levels than the ethanol group, $p = 0.06$, and the corticosterone group had lower levels than the water group, $p < 0.001$. At the zero time point the ethanol group had lower levels than the water group, $p = 0.001$. At 45 minutes after the test among the rats treated in adolescence and tested immediately corticosterone treated rats had lower levels than both the water and ethanol groups, $p < 0.001$, and ethanol and water groups did not significantly differ from one another (see Figure 22). For rats treated in adolescence and tested immediately, at 90 minutes after the FST, the corticosterone group had lower levels than the ethanol group, $p = 0.03$ (see Figure 22).

For the rats treated in adolescence and tested several weeks later, there was no treatment effect. The corticosterone levels decreased with time after the FST, $F(2,26) = 31.30$, $p < 0.001$ (see Figure 22).

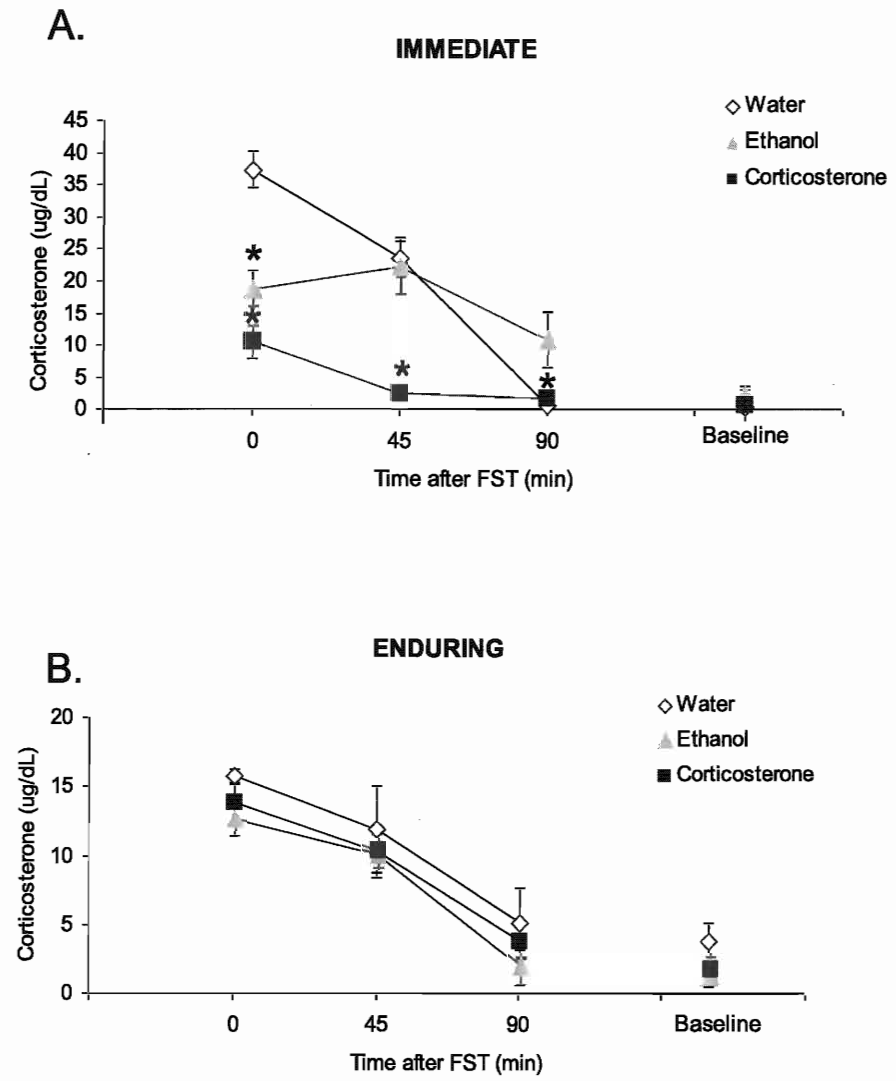
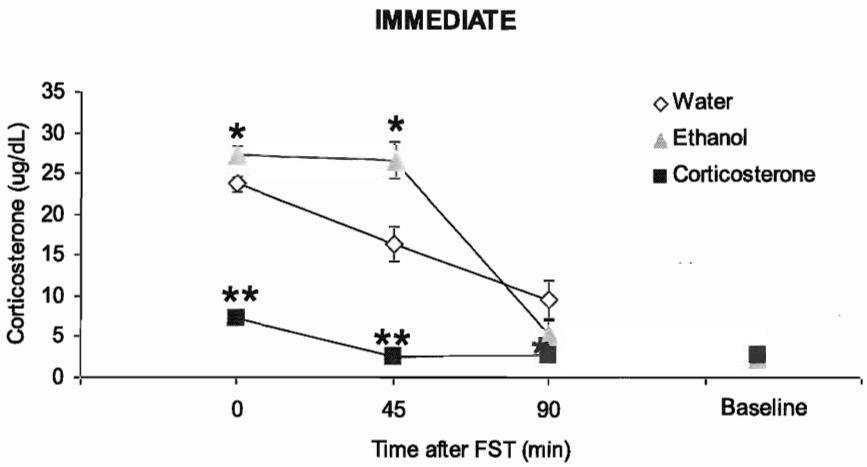


Figure 22. The means for the plasma concentrations of corticosterone experienced after the FST for those adolescent treated rats tested A. for immediate effects and B. for enduring effects. Error bars represent the SEM. * Indicates significantly different from the water group at that time point, $p = 0.03$ or less.

For adults there was a significant time point by treatment by time of testing interaction on corticosterone levels, $F(4,56) = 5.35$, $p = 0.001$. This was followed up with repeated measures ANOVAs of corticosterone concentrations at the three measured time points after the FST for each time of testing group. For the rats treated as adults and tested immediately, the interaction between time point and treatment was significant, $F(4,30) = 13.92$, $p < 0.001$. This was followed up with ANOVAs of corticosterone concentration at each time point. Post hoc analyses showed that at the zero time point, immediately after removal from the FST, the corticosterone group had lower corticosterone levels than the ethanol group, $p < 0.001$, and the corticosterone group had lower levels than the plain water group, $p < 0.001$. The ethanol group also had higher levels than the plain water group $p = 0.03$. At 45 minutes after the FST the corticosterone group had significantly lower levels of corticosterone than the water group, and the water group had significantly lower levels than the ethanol group, $p < 0.001$ for each comparison. For the adult treated rats tested immediately at 90 minutes after the FST the corticosterone group had lower levels of corticosterone than the water group, $p = 0.02$, and no other groups differed from one another (see Figure 27). For the rats treated as adults and tested several weeks later there was a significant effect of time point, $F(2,26) = 46.90$, $p < 0.001$, such that corticosterone levels decrease with time after the FST, but there was no effect of treatment (see Figure 23).

CORTICOSTERONE AFTER FST ADULT TREATED

A.



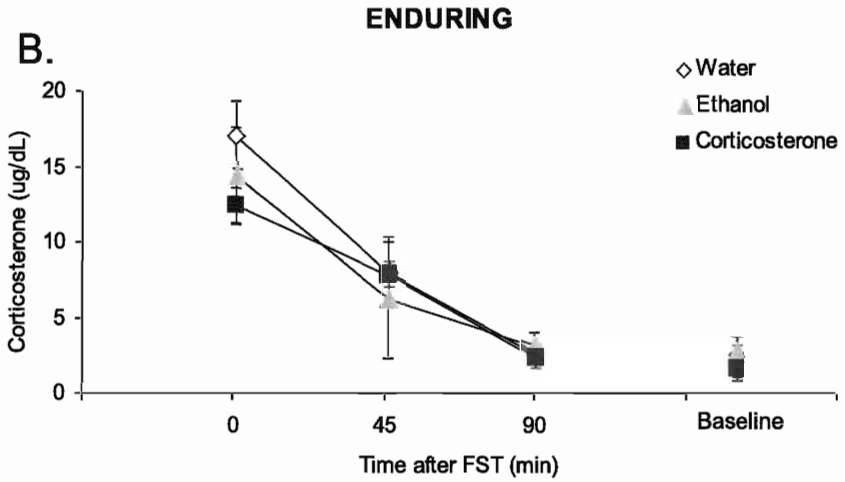


Figure 23. The means for the plasma concentrations of corticosterone experienced after the FST for those adult rats tested A. for immediate effects and B. for enduring effects. Error bars represent the SEM. * Indicates significantly different from water at that time point, $p = 0.03$ or less. ** Indicates significantly different from water and ethanol at that time point, $p < 0.000$

Baseline Corticosterone

For the adolescent treated rats tested either immediately or several weeks later, there was no effect of treatment on baseline corticosterone, $F(2,15) = 1.88$, $p = 0.19$ for immediately tested and $F(2,13) = 2.35$, $p = 0.13$ for those tested several weeks later (see Figure 22). For the adult treated rats there was no effect of treatment, $F(2,17) = 0.05$, $p = 0.95$ for those tested immediately and $F(2,13) = 0.40$, $p = 0.68$ for those tested several weeks later (see Figure 23).

Discussion for Experiment 2

The main findings from Experiment 2 were that despite demonstrating effects on weight, corticosterone treatment was not sufficient to produce effects on the behavior of either adult or adolescent treated rats regardless of whether the rats were tested soon after treatment or weeks later. Corticosterone treatment was sufficient to dampen the HPA response to a novel stressor when tested soon after treatment regardless of the age at which treatment was administered. However, there were no effects of corticosterone treatment on HPA response when tested weeks after treatment regardless of the age at which treatment occurred.

Weight

Corticosterone treatment inhibited weight gain when administered either in adolescence or in adulthood. In both cases, corticosterone treated rats continued to weigh less than those without corticosterone one week after the period of administration was over, but after two weeks there were no differences in weight. For rats treated in adolescence ethanol treatment also decreased weight. Because ethanol has more calories than regular water, this decrease in weight was the opposite effect than expected. This effect on weight may be an indication that drinking ethanol is aversive for adolescents; it has been shown that adolescents mount a corticosterone response to ethanol exposure and that response is greater in adolescents than in adults (Silveri & Spear, 2004). As discussed previously, corticosterone has been shown to decrease weight; therefore the effect of ethanol on weight may be the result of a corticosterone response to ethanol consumption. That this effect is observed in the adolescents but not the adults may be the result of different responses to ethanol at differing ages, or it may be that the smaller dose ingested by the adults was insufficient to produce a weight difference.

All groups recovered from the effects of corticosterone treatment such that their weight was not different from untreated rats of the corresponding age group two weeks after the end of treatment, which suggests that this method of administration is not as potent as injection, for which weight differences persisted for three weeks. In those rats that received injections the weight differences were maintained for at least three weeks, despite the fact that the injected dose was lower than the doses ingested by rats administered corticosterone in their drinking water. The injected dose used in Experiment 1 has been shown to produce levels of corticosterone at about 2100 ng / mL for up to four hours after injection and decreased to around 450 ng / mL by the next day (Sousa et al., 1998), and the concentration of corticosterone given in the drinking water here, 400 μg / mL, has been shown to maintain a consistent plasma concentration of corticosterone at around 200 ng / mL, which was shown to be significantly increased from basal levels of vehicle treated controls (Magarinos et al., 1998). Therefore despite the fact that the rats are taking in a greater dose of corticosterone, the effect is a much lower plasma concentration though it is maintained as consistently higher than vehicle treated controls. The difference in plasma concentrations may be due to different rates of absorption from a subcutaneous injection as compared to ingestion, the former getting absorbed into the bloodstream underneath the skin and the latter from the gut, or possibly the rate of intake is the key factor. Injected corticosterone is delivered once a day, but corticosterone administered in the drinking water is delivered to the animal several times a day.

Intake and Doses

The rats that were treated only with ethanol in their drinking water ingested a similar dose of ethanol to the corticosterone and ethanol treated group of corresponding age. However the ethanol treated groups are not good control groups to use for the purpose of interpreting the

effects of corticosterone treatment because the effects of ethanol on weight indicate that ethanol consumption may be aversive, in that it altered physiology and metabolism. Therefore it is important to consider the comparison both to the ethanol treated rats and the rats that received plain water. The adolescent treated groups ingested a higher dose of both ethanol and corticosterone at the beginning of the period of administration. This difference is mainly the result of their weight, rats were consuming roughly the same amount of ethanol and corticosterone throughout the period of administration but the adolescence started out at a much lower weight.

Depressive Behavior

Neither corticosterone nor ethanol had any effect on depressive behavior in the FST regardless of the age at which the rats were treated or the time at which they were subsequently tested. The duration of treatment for Experiment 2 was the same as it was in Experiment 1, and therefore may simply be insufficient to produce an effect of corticosterone in the FST, as discussed previously (reviewed in Sterner & Kalynchuk, 2010). However, unlike in Experiment 1, the control groups here are completely without stressor experience. They were not even handled outside of regular cage cleanings during the period of administration. The rats during this experiment were also exposed to more corticosterone over the course of treatment, but this exposure was a little at a time. By administering corticosterone in the drinking water the control over the rate of administration is given to the rat. Therefore it is unlikely any of these rats ingested enough of their drinking water to get a very high dose of corticosterone at one instance.

Corticosterone administered in drinking water has been shown to have varying effects on basal corticosterone levels such that it increased basal corticosterone when measured immediately after treatment (Magarinos et al., 1998), but not when measured two weeks later

(Gourley & Taylor, 2009). In general administration methods such as corticosterone dissolved in drinking water or implanted corticosterone pellets or pumps have been shown to have a smaller effect on the plasma concentrations of corticosterone than injections (reviewed in Sterner & Kalynchuk, 2010). These administration methods expose the rats to a constant but small elevation in corticosterone, which is not a good representation of what a rat undergoing chronic stress would experience. Acute stressors induce a large release of corticosterone that is brief in duration because negative feedback will quickly slow down its release. Even when stressors are presented chronically they involve a rise in corticosterone with a subsequent return to baseline. Therefore administering corticosterone in drinking water is not a good model for understanding the effects of stress that are the result of corticosterone exposure.

Adolescent treated rats that were tested several weeks after treatment spent more time immobile, less time swimming or climbing and had a lower latency to become immobile than those adolescent treated rats that were tested immediately after treatment regardless of treatment group. Because these rats demonstrated no effect of treatment on behavior at either one of the times for testing, these differences are likely indicative of an age effect. The rats treated in adolescence and tested immediately after treatment were tested during adolescence, postnatal day 47, but those tested several weeks after treatment were tested during adulthood, postnatal day 71. Therefore these results indicate that adult rats in general have a higher level of depressive behavior on the FST. The same effect was observed in Experiment 1 as well; the rats tested several weeks after treatment spent more time immobile than those tested immediately after treatment. This finding is consistent with other studies that have shown adolescents to spend less time immobile than adults (Hefner & Holmes, 2007; Pechnick et al., 2008). These findings

demonstrate the importance of including age matched controls when studying the effects of exposure to stress or corticosterone during adolescence.

HPA Response to a Stressor for Adolescent Treated Rats

As predicted, the corticosterone treated rats tested immediately after treatment produced a smaller HPA response to a stressor, lower corticosterone concentrations after the FST, than those treated with ethanol or water regardless of the age at which the treatment was administered, similar to the results observed in Experiment 1. Additionally, rats treated with ethanol during adolescence and tested immediately after treatment produced a smaller initial corticosterone release after the FST than those treated with plain water. However, adult rats treated with ethanol and tested immediately after treatment displayed enhanced corticosterone response to the FST as compared to those treated with plain water. Additionally, for the rats treated at either age and tested several weeks after treatment, treatment produced no effect on HPA response to a stressor.

As in Experiment 1, when rats were tested immediately after treatment the corticosterone response to a stressor was dampened. This is likely the result of the ongoing negative feedback produced by exposure to exogenous corticosterone. These results are consistent with other studies that have found the HPA response dampened when measured immediately after corticosterone treatment both when administration is given by injection (Johnson et al., 2006) and by dissolving corticosterone in the drinking water (Pung et al., 2003; Gourley & Taylor, 2009). This indicates that corticosterone treatment in the drinking water is sufficient to activate negative feedback decreasing the production of corticosterone in the adrenal gland for some time after its removal from the drinking water.

Because ethanol treatment during adolescence had a dampening effect on the HPA response to a stressor, administration of corticosterone when dissolved with ethanol in the

drinking water is not well suited for studies involving adolescents. Chronic exposure to ethanol can increase basal concentrations of corticosterone, but this result was found using a higher concentration of ethanol, 5%, than that used in this experiment, 2.5%, and a longer duration, 28 days, than that used here, 16 days (Rasmussen et al., 2000). Other studies have shown that chronic exposure to ethanol can blunt the HPA response to stress in adults both with humans and rodents (reviewed in Prendergast & Little, 2007). Therefore these results are consistent with that literature, and indicate that a relatively low dose of ethanol is required to blunt the HPA response to a stressor when the ethanol is provided during adolescence. It has also been observed that ethanol exposure induces an increase in the plasma concentration of corticosterone (Silveri & Spear, 2004), and therefore the effects of ethanol exposure observed here may be the result of exposure to corticosterone.

However ethanol treatment during adulthood had the opposite effect of ethanol treatment during adolescence on the HPA response to a stressor. This may be the result of a difference the effects of ethanol on a developing system compared to a mature system. However, it must also be considered that the adolescents consumed a larger dose of ethanol, and therefore it is unclear whether the discrepancy between the effects of treatment at these ages is the result of the difference in dose or age. Other studies have shown a blunted HPA response after chronic exposure to ethanol (reviewed in Prendergast & Little, 2007), and that ethanol can increase basal corticosterone concentrations (Rasmussen et al., 2000). However, as mentioned previously these studies use larger concentrations and longer durations of administration, therefore the discrepancy may be the result of these differences in exposure. Additionally one study has observed that with early adolescents, postnatal day 34 and young adults, postnatal day 60, six exposures to a small dose of corticosterone (2.2-2.6 g/kg) can increase the corticosterone

response to swim stress (Silveri & Spear, 2004). The dose used in that study is closest to the dose ingested by the adults, and the effect is opposite what is observed in studies using larger doses (Rasmussen et al., 2000), therefore it may be that dose and not age is the relevant factor. Therefore it is plausible that a shorter duration and lower dose when administered during adulthood may produce an increase in corticosterone response to stressors.

The lack of an enduring effect of corticosterone on HPA response may be the result of the nature of administration and its inability to sufficiently activate GRs. Corticosterone administration that does not produce a very high plasma concentration of corticosterone, such as administration in drinking water, may not be able to activate many glucocorticoid receptors (GRs). Because mineralocorticoid receptors (MRs) have a higher affinity for corticosterone, at low concentrations of corticosterone most MRs are occupied (89.5%) and few GRs are (15%) (Reul & de Kloet, 1985; Spencer et al., 1990). Therefore administering corticosterone in the drinking water is likely to increase MR activation, but not likely to have much of an effect on GR activation. Because MRs are activated at basal concentrations of corticosterone and GRs are activated after high concentrations of corticosterone, such as those experienced after a stressor, it is thought that the effects of stress that are corticosterone related are the result of the activation of GRs (reviewed in de Kloet et al., 1998). In Experiment 1 corticosterone treatment by injection during adolescence produced an increased HPA response when tested several weeks after treatment, and here there was no effect of treatment despite the fact that the rats were ingesting larger doses of corticosterone over the same period of time at the same ages. The most likely explanation for this discrepancy is that exposure to a sufficiently high concentration of corticosterone that adequately activates GRs is required to produce enduring effects on the HPA response.

General Discussion

The most definitive conclusion that can be drawn from these two experiments is that when studying adolescents one needs to consider carefully the effects of every manipulation and carefully design control groups to account for possible confounds. Corticosterone displayed no effects on depressive or anxiety-like behavior regardless of age at which the treatment was administered, time at which the rats were tested, or method of administration used. Exposure to exogenous corticosterone administered during adolescence had an enduring effect to increase the HPA response to a stressor when administered by injections, but not when administered in the drinking water and not when administered in adulthood. Exposure to ethanol during either adulthood or adolescence can affect the HPA axis. In adolescence ethanol dampened the HPA response to a stressor, and in adulthood ethanol increased the HPA response to a stressor. However, the adolescents ingested a higher dose of ethanol during the majority of the administration period; therefore it remains unclear whether the different effects are a result of age differences or dose differences.

The fact that ethanol can affect the HPA demonstrates that administration of corticosterone with ethanol dissolved in drinking water is a poor model for studying the effects of corticosterone on the HPA, because the effects of ethanol and corticosterone are not easily distinguished. However administering corticosterone by injections involve a stressor to which adolescents may be uniquely sensitive, and therefore corticosterone injections are a poor model to use when studying adolescents. A better way to study the effects of corticosterone exposure during adolescence may be to induce endogenous release of corticosterone with a stressor.

It is also important to consider that the stress response is much more complex than the eventual release of corticosterone from the adrenal gland. Corticosterone release is only one step

in the response of the HPA axis to a stressor; the hypothalamus becomes activated and releases both corticotrophin releasing hormone and arginine vasopressin and the pituitary will release adrenocorticotropic hormone which in turn signals the adrenals to release corticosterone (reviewed in Herman & Cullinan, 1997). When a stressor is experienced all of these hormones are released and have their own unique effects on the brain and body. In addition to the HPA axis other systems also respond to stressors, for example the noradrenergic system releases catecholamines in response to a stressor to increase arousal (Morilak et al., 2005). Endogenous opiates are also released in response to a stressor, which can induce analgesia (reviewed in McEwen & Sapolsky, 1995). The response to a stressor involves much more than corticosterone release, and therefore experiments that examine the effects of corticosterone administration, such as those described in this thesis, only account for one facet of the stress response.

References

Abel, E. L. (1994). Behavioral and physiological effects of different water depths in the forced swim test. Physiology & Behavior, 56(2), 411-414.

Adamec, R.E., Blundell, J., & Collins, A. (2001). Neural plasticity and stress induced changes in defense in the rat. Neuroscience and Biobehavioral Revews, 25, 721-744.

Adamec, R., Head, D., Blundell, J., Burton, P., & Berton, O. (2006). Physiology & Behavior, 88, 12-29.

Adamec, R., Walling, S., & Burton, P. (2004). Long-lasting, selective, anxiogenic effects of feline predator stress in mice. Physiology & Behavior, 83, 401-410.

Akirav, I., Kozenicky, M., Tal, D., Sandi, C., Venero, C., & Richter-Levin, G. (2004). A facilitative role for corticosterone in the acquisition of a spatial task under moderate stress. Learning & Memory, 11, 188-195.

Alonso, G. (2000). Prolonged corticosterone treatment of adult rats inhibits the proliferation of oligodendrocyte progenitors present throughout white and gray matter regions of the brain. Glia, 31, 219-231.

Armario, A., Gavalda, A., & Marti, O. (1988). Forced swimming test in rats: effect of desipramine administration and the perdiod of exposure to the test on struggling behavior, swimming, immobility and defecation rate. European Journal of Pharmacology, 158, 207-212.

Armario, A., Gil, M., Marti, J., Pol, O., & Balasch, J. (1991). Influence of various acute stressors on the activity of adult male rats in a holeboard and in the forced swim test. Pharmacology, Biochemistry & Behavior, 39, 373-377.

Avital, A. & Richter-Levin, G. (2005). Exposure to juvenile stress exacerbates the behavioural consequences of exposure to stress in the adult rat. International Journal of Neuropsychopharmacology, 8, 163-173.

Bhatnagar, S. & Dallman, M. (1998). Neuroanatomical basis for facilitation of hypothalamic-pituitary-adrenal responses to a novel stressor after chronic stress. Neuroscience, 84, 1025-1039.

Bodnoff, S. A., Humphreys, A. G., Lehman, J. C., Diamond, D. M., Rose, G. M., & Meany, M. J. (1995). Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. The Journal of Neuroscience, 15(1), 61-69.

Bondi, C. O., Rodriguez, G., Gould, G. G., Frazer, A., & Morilak, D. A. (2008). Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. Neuropsychopharmaology, 33, 320-331.

Bowman, R.E., Micik, R., Gautreaux, C., Fernandez, L., & Luine, V.N. (2009). Sex-dependent changes in anxiety, memory, and monoamines following one week of stress. Physiology & Behavior, 97, 21-29.

Calvo, N. & Volosin, M. (2001). Glucocorticoid and mineralocorticoid receptors are involved in the facilitation of anxiety-like response induced by restraint. Neuroendocrinology, 73, 261-271.

Cancela, L. M., Rossi, S., & Molina, V. A. (1991). Effect of different restraint schedules on the immobility in the forced swim test: modulation by an opiate mechanism. Brain Research Bulletin, 26, 671-675.

Cerqueira, J. J., Pego, J. M., Taipa, R., Bessa, J. M., Almeida, O. F. X., & Sousa, N. (2005). Morphological correlates of corticosteroid-induced changes in prefrontal cortex-dependent behaviors. The Journal of Neuroscience, 25, 7792-7800.

Cerqueira, J. J., Taipa, R., Uylings, H. B. M., Almeida, O. F. X., & Sousa, N. (2007). Specific configuration of dendritic degeneration in pyramidal neurons of the medial prefrontal cortex induced by differing corticosteroid regimens. Cerebral Cortex, 17, 1998-2006.

Chalmers, D. T., Kwak, S. P., Mansour, A., Akil, H., & Watson, S. J. (1993). Corticosteroids regulate brain hippocampal 5-HT1A receptor mRNA expression. The Journal of Neuroscience, 13(3), 914-923

Cole, M. A., Kalman, B. A., Pace, T. W. W., Topczewski, F., Lowrey, M. J. & Spencer, R. L. (2000). Selective blockade of the mineralocorticoid receptor impairs hypothalamic-pituitary-adrenal axis expression of habituation. Journal of Neuroendocrinology, 12, 1034-1042.

Collins, A., Hill, L. E., Chandramohan, Y., Whitcomb, D., Droste, S. K., & Reul, J. M. H. (2009). Exercise improves cognitive responses to psychological stress through enhancement of epigenetic mechanisms and gene expression in the dentate gyrus. PLos ONE 4(1), e4330.

Cordero, M. I., Merino, J. J., & Sandi, C. (1998). Correlational relationship between shock intensity and corticosterone secretion on the establishment and subsequent expression of contextual fear conditioning. Behavioral Neuroscience, 112(4), 885-891.

Dallman, M. F. (1984). Viewing the ventromedial hypothalamus from the adrenal gland. American Journal of Physiology – Regulatory, Integrative and Comparative Physiology, 246, 1-12.

David, D. J., Samuels, B. A., Rainer, Q., Wang, J. W., Marstellar, D., Mendez, I., Drew, M., Craig, D. A., Guiard, B. P., Guilloux, J.P., Artymyshyn, R. P., Gardier, A. M., Gerald, C., Antonijevic, I. A., Leonardo, E. D., & Hen, R. (2009). Neurogenesis-dependent and - independent effects of fluoxetine in an animal model of anxiety/depression. Neuron, 62, 479-493.

Davis, L., Barlow, D. H., & Smith, L. (2010a). Comorbidity and the treatment of principle anxiety disorders in a naturalistic sample. Behavior Therapy, 41, 296-305.

Davis, M., Walker, D. L., Miles, L., & Grillon, C. (2010b). Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. Neuropsychopharmacology, 35 (1), 1-31.

Dean, F. & Matthews, S. G., (1999). Maternal dexamethasone treatment in late gestation alters glucocorticoid and mineralocorticoid receptor mRNA in the fetal guinea pig brain. Brain Research, 253-259.

De Kloet, E. R., Vreugdenhil, E., Oitzl, M. S., & Joels, M. (1998). Brain corticosteroid receptor balance in health and disease. Endocrine Reviews, 19(3), 269-301.

Detke, M. J., Rickels, M., & Lucki, I. (1995). Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology, 121, 66-72.

Doremus-Fitzwater, T. L., Varlinskaya, E. I., & Spear, L. P. (2004). Age-related differences in elevated plus maze behavior between adolescent and adult rats. Annals of New York Academy of Sciences, 1021, 427-430.

Doremus-Fitzwater, T. L., Varlinskaya, E. I., & Spear, L. P. (2006). Factor analysis of elevated plus-maze behavior in adolescent and adult rats. Pharmacology, Biochemistry, and Behavior, 83, 570-577.

Doremus-Fitzwater, T. L., Varlinskaya, E. I., & Spear, L. P. (2009a). Effects of pretest manipulation on elevated plus-maze behavior in adolescent and adult male and female sprague-dawley rats. Pharmacology, Biochemistry, and Behavior, 92, 413-423.

Doremus-Fitzwater, T. L., Varlinskaya, E. I., & Spear, L. P. (2009b). Social and non-social anxiety in adolescent and adult rats after repeated restraint. Physiology & Behavior, 97, 484-494.

Echandia, R. E. L., Gonzalez, A. S., Cabrera, R., & Fracchia, L. N. (1988). A further analysis of behavioral and endocrine effects of unpredictable chronic stress. Physiology & Behavior, 43, 789-795.

Galea, L. A. M., McEwen, B. S., Tanapat, P., Deak, T., Spencer, R. L., & Dhabhar, F. S. (1997). Sex differences in dendritic atrophy of CA3 pyramidal neurons in response to chronic restraint stress. Neuroscience, 81(3), 689-697.

Gamiero, G. H., Gamiero, P. H., Andrade, A. d. S., Pereira, L. F., Arthuri, M. T., Marcondes, F. K., & Veiga, M. C. F. d. A. (2006). Nociception- and anxiety-like behavior in rats submitted to different periods of restraint stress. Physiology & Behavior, 87, 643-649.

Gannon, M. N. & McEwen, B. S. (1990). Calmodulin involvement in stress- and corticosterone-induced down-regulation of cyclic AMP-generating systems in brain. Journal of Neurochemistry, 55(1), 277-284.

Giedd, J. N., Keshavan, M., & Paus, T. (2008). Why do many psychiatric disorders emerge during adolescence? Nature Reviews Neuroscience, 9(12), 947-957.

Goliszek, A. G., Crawford, G. E., Lawrence, H. S., Bennett, J., Williams, F., & Hurley, S. L. (1996). Effects of prepubertal stress on subsequent ACTH response to novel stress and CRH in male vs. female rats. Stress Medicine, 12, 199-204.

Gomez, F., Houshyar, H., & Dallman, M. F. (2002). Marked regulatory shifts in gonadal, adrenal, and metabolic system responses to repeated restraint stress occur within a 3-week period in pubertal male rats. Endocrinology, 143, 2852-2862.

Gourley, S. L. & Taylor, J. R. (2009). Recapitulation and reversal of a persistent depression-like syndrome in rodent. Current Protocols of Neuroscience, 9.32, 1-14.

Gourley, S. L., Wu, F. J., Kiraly, D. D., Ploski, J. E., Kedves, A. T., Duman, R. S., & Taylor, J. R. (2008). Regionally specific regulation of ERK MAP kinase in a model of antidepressant-sensitive chronic depression. Biological Psychiatry, 63, 353-359.

Gregus, A., Wintink, A. J., Davis, A. C., & Kalynchuk, L. E. (2005) Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. Behavioural Brain Research, 156, 105-114

Grissom, N., Iyer, I., Vining, C., & Bhatnagar, S. (2007). The physical context of previous stress exposure modifies hypothalamic-pituitary-adrenal responses to a subsequent homotypic stress. Hormones & Behavior, 51, 95-103.

Hefner, K. & Holmes, A. (2007). Ontogeny of fear-, anxiety- and depression-related behavior across adolescence in C57BL/6J mice. Behavioural Brain Research, 176, 210-215.

Herman, J. P. & Cullinan, W. E. (1997). Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Neurocircuitry of stress, 20, 78-84

Herrmann, M., Henneicke, H., Street, J., Modzelewski, J., Kalak, R., Buttgereit, F., Dunstan, C. R., Zhou, H., & Seibel, M. J. (2009). The challenge of continuous exogenous glucocorticoid administration in mice. Steroids, 74, 245-249.

Imhof, J. T., Coelho, Z. M. I., Schmitt, M. L., Morato, G. S., & Carobrez, A. P. (1993). Influence of gender and age on performance of rats in the elevated plus maze apparatus. Behavioural Brain Research, 56,177-180.

Isgor, C., Kabbaj, M., Akil, H., & Watson, S. J. (2004). Delayed effects of chronic variable stress during peripubertal-juvenile period on hippocampal morphology and on cognitive and stress axis functions in rats. Hippocampus, 14, 636-648.

Johnson, S. A., Fournier, N. M., & Kalynchuk, L. E. (2006). Effect of different doses of corticosterone on depression-like behaviour and HPA axis to a novel stressor. Behavioural Brain Research, 168, 280-288

Kalynchuk, L. E., Gregus, A., Bourdreau, D. & Perrot-Sinal, T. S. (2004). Corticosterone increases depression-like behavior, with some effects on predator odor-induced defensive behavior, in male and female rats. Behavioral Neuroscience, 118(6), 1365-1377.

Kavushansky, A., Ben-Shachar, D., Richter-Levin, G., & Klein, E. (2009). Physical stress differs from psychosocial stress in the pattern and time-course of behavioral responses, serum corticosterone and expression of plasticity-related genes in the rat. Stress, 12(5), 412-425.

Klein, Z. A., Padow, V. A., Romeo, R. D. (2010). The effects of stress on play and home cage behaviors in adolescent male rats. Developmental Psychobiology, 52(1), 62-71

Kompagne, H., Bardos, G., Szenasi, G., Gacsalyi, I., Harsing, L. G., & Levay, G. (2008). Chronic mild stress generates clear depressive but ambiguous anxiety-like behaviour in rats. Behavioural Brain Research, 193, 311-314.

Korte, S. M., De Boer, S. F., De Kloet, E. R., & Bohus, B. (1995). Anxiolytic-like effects of selective mineralocorticoid and glucocorticoid antagonists on fear-enhanced behavior in the elevated plus maze. Psychoneuroendocrinology, 20(4), 385-394.

Lee, B., Shim, I., Lee, H. J., Yang, Y., & Hahm, D. H. (2009). Effects of acupuncture on chronic corticosterone-induced depression-like behavior and expression of neuropeptide Y in the rats. Neuroscience Letters. 453, 151-156.

Lepsch, L. B., Gonzola, L. A., Magro, F. J., Delucia, R., Scavone, C., & Planeta, C. S. (2005). Exposure to chronic scress increases the locomotor response to cocaine and the basal levels of corticosterone in adolescent rats. Addiction Biology, 10(3), 251-256.

Leussis, M. P., Lawson, K., Stone, K., & Andersen, S. L. (2008). The enduring effects of an adolescent social stressor on synaptic density, part II: Poststress reversal of synaptic loss in the cortex by adinazolam and MK-801. Synapse, 62, 185-192.

Lukkes, J. D., Mokin, M. V., Scholl, J. L., & Forster, G. L. (2009). Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior and altered hormonal stress responses. Hormones & Behavior, 55, 248-256.

Lunga, P. & Herbert, J. (2004). 17β-Oestradiol modulated glucocorticoid, neural and behavioural adaptations to repeated restraint stress in female rats. Journal of Neuroendocrinology, 16, 76-785.

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nature Reviews, 10, 434-446.

Macri, S., Adriani, W., Chiarotti, F., & Laviola, G. (2002). Risk taking during exploration of a plus-maze is greater in adolescent than in juvenile or adult mice. Animal Behaviour, 64, 541-546.

Magarinos, A. M., Orchinick, M., McEwen, B. S. (1998). Morphological changes in the hippocampal CA3 region induced by non-invasive glucocorticoid administration: a paradox. Brain Research, 809, 314-318.

Maniam, J. & Morris, M. J. (2010). Palatable cafeteria diet ameliorates anxiety and depression-like symptoms following an adverse early environment. Psychoneuroendocrinology, 35 (5), 717-728.

Marks, W., Fournier, N. M., & Kalynchuk, L. E. (2009). Repeated exposure to corticosterone increases depression-like behavior in two different versions of the forced swim test without altering nonspecific locomotor activity or muscle strength. Physiology & Behavior, 98, 67-72.

Maslova, L. N., Bulygina, V. V., & Markel, A. L. (2002). Chronic stress during prepubertal development: immediate and long-lasting effects on arterial blood pressure and anxiety-related behavior. Psychoneuroendocrinology, 27, 549-561.

Mathews, I. Z., Wilton, A., Styles, A., & McCormick, C. M. (2008). Heightened neuroendocrine function in males to a heterotypic stressor and increased depressive behaviour in females after adolescent social stress in rats. Behavior and Brain Research, 190, 33-40.

Matuszewich, L., Karney, J. J., Carter, S. R., Janasik, S. P., O'Brien, J. L., & Friedman, R. D. (2007). The delayed effects of chronic unpredictable stress on anxiety measures. Physiology & Behavior, 90, 674-681.

McCormick, C. M., & Mathews, I. Z. (2007). HPA function in adolescence: Role of sex hormones in its regulation and the enduring consequences of exposure to stressors. Pharmacology Biochemistry Behavior, 86, 220-233.

McCormick, C. M., Mathews, I. Z., Thomas, C., & Waters, P. (2010). Investigations of HPA function and the enduring consequences of stressors in adolescence in animal models. Brain and Cognition, 72(1), 73-85

McCormick, C. M., Merrick, A., Secen, J., & Helmreich, D. L. (2007). Social instability in adolescence alters the central and peripheral hypothalamic-pituitary-adrenal responses to a repeated homotypic stressor in male and female rats. Journal of Neuroendocrinology, 19, 16-126.

McCormick, C. M., Robarts, D., Gleason, E., & Kelsey, J. E. (2004). Stress during adolescence enhances locomotor sensitization to nicotine in adulthood in female, but not male, rats. Hormones & Behavior, 46, 458-466.

McCormick, C. M., Robarts, D., Kopeikina, K., & Kelsey, J. E. (2005). Long-lasting, sex-and age-specific effects of social stress on corticosterone responses to restraint and locomotor responses to psychostimulants in rats. Hormones & Behavior, 48, 64-74.

McCormick, C. M., Smith, C., & Mathews, I. Z. (2008). Effects of chronic social stress in adolescence on anxiety and neuroendocrine response to mild stress in male and female rats. Behavioural and Brain Research, 187, 228-238.

McEwen, B. S. & Sapolsky, R. M. (1995) Stress and cognitive function. Current Opinion in Neurobiology, 5, 205-216.

Mitra, R. & Sapolsky, R. M. (2008). Acute corticosterone treatment is sufficient to induce anxiety and amygdaloid dendritic hypertrophy. Proceedings of the National Academy of the Sciences, 105(14), 5573-5578.

Mitra, R., Jadhav, S., McEwen, B. S., Vyas, A., & Chattarji, S. (2005). Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. Proceedings of the National Academy of the Sciences, 102(26). 9371-9376.

Molina, V. A., Heyser, C. J., & Spear, L. P. (1994). Chronic variable stress or chronic morphine facilitates immobility in a forced swim test: reversal by naloxone. Psychopharmacology, 114, 433-440.

Morilak, D. A., Barrera, G., Echevarria, D. J., Garcia, A. S., Hernandez, A., Ma, S., & Petre, C. O. (2005). Role of brain norepinephrine in the behavioral response to stress. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 29, 1214-1224.

Munoz- Abellan, C., Armario, A., & Nadal, R. (2010). Do odors from different cats induce equivalent unconditioned and conditioned response in rats? Physiology & Behavior, 99 (3), 388-394

Munoz-Abellan, C., Daviu, N., Rabasa, C., Nadal, R., & Armario, A. (2009). Cat odor causes long-lasting contextual fear conditioning and increased pituitary-adrenal activation, without modifying anxiety. Hormones & Behavior, 56, 465-471.

Murray, F., Smith, D. W., & Hutson, P. H. (2008). Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice. European Journal of Pharmacology, 583, 115-127.

Nacher, J., Pham, K., Gil-Fernandez, V., & McEwen, B. S. (2004). Chronic restraint stress and chronic corticosterone treatment modulate differentially the expression of molecules related to structural plasticity in the adult rat piriform cortex. Neuroscience, 126, 503-509.

Noschang, C. G., Pettenuzzo, L. F., Toigo, E. v. P., Andreazza, A. C., Krolow, R., Fachin, A., Avila, M. C., Arcego, D., Crema, L. M., Diehl,L. A., Goncalvez, C. A., Vendite, D., & Dalmaz, C. (2009). Sex-specific differences on caffeine consumption and chronic stress-induced anxiety-like behavior and DNA breaks in the hippocampus. Pharmacology, Biochemistry and Behavior, 94, 63-69.

Novak, C. M., Parfitt, D. B., Sisk, C. L., & Smale, L. (2007). Associations between behavior, hormonse, and Fos responses to novelty differ in pre- and post-pubertal grass rats. Physiology & Behavior, 90, 125-132.

Overstreet, D. H. & Griebel, G. (2004). Antidepressant-like effects of CRF1 receptor antagonist SSR125543 in an animal model of depression. European Journal of Pharmacology, 497, 49-53.

Overmier, J. B., & Murison, R. (1991). Juvenile and adult footshock stress modulate later adult gastric pathophysiological reactions to restraint stresses in rats. Behavioral Neuroscience, 105, 246-252.

Parker, K. J., Schatzberg, A. F., & Lyons, D. M. (2003). Neuroendocrine aspects of hypercortisolism in major depression. Hormones & Behavior, 43, 60-66.

Pechnick, R. N., Bresee, C. J., Manalo, C. M., & Polan, R. E. (2008). Comparison of the effects of desmethylimipramine on behavior in the forced swim test in peripubertal and adult rats. Behavioural Pharmacology, 19(1), 81-85.

Peeters, F., Nicolson, N. A., Berkof, J., Delespaul, P., deVries, M.(2003). Effects of Daily Events on Mood States in Major Depressive Disorder. Journal of Abnormal Psychology, 112(2), 203-211.

Pego, J. M., Morgado, P., Pinto, L. G., Cerqueira, J. J., Almeida, O. F. X., & Sousa, N. (2008). Dissociation of the morphological correlates of stress-induced anxiety and fear. European Journal of Neuroscience, 27, 1503-1516.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of Neuroscience Methods, 14, 149-167.

Pohl, J., Olmstead, M. C., Wynne-Edwards, K. E., & Menard, J. L. (2007). Repeated exposure to stress across the childhood-adolescent period alters rats' anxiety- and depression-like behaviors in adulthood: The importance of stressor type and gender. Behavioral Neuroscience, 121, 462-474.

Pol, O., Campmany, L., Gil, M., & Armario, A. (1992). Behavioral and neurochemical changes in response to acute stressors: influence of previous chronic exposure to immobilization. Pharmacology Biochemistry and Behavior, 42, 407-412.

Porsolt, R. D., Anton, G., Blavet, N., & Jalfre, M. (1978). Behavioural despair in rats: a new model sensitive to antidepressant treatments. European Journal of Pharmacology, 47, 379-391.

Porsolt, R. D., Le Pichon, M., & Jalfre, M. (1977). Depression: a new animal model sensitive to antidepressant treatments. Nature, 266, 730-731.

Prendergast, M. A. & Little, H. J. (2007). Adolescence, glucocorticoids and alcohol. Pharmacology, Biochemistry and Behavior, 86, 234-245.

Pung, T., Zimmerman, K., Klein, B., & Ehrich, M. (2003). Corticosterone in drinking water: altered kinetics of a single oral dose of corticosterone and concentrations of plasma sodium, albumin, globulin, and total protein. Toxicology and Industrial Health, 19, 171-182.

Rachman, I. M., Unnerstall, J. R., Pfaff, D. W., & Cohen, R. S. (1998). Estrogen alters behavior and forebrain c-fos expression in ovariectomized rats subject to the forced swim test. Neurobiology, 95, 13941-13946.

Rasmussen, D. D., Boldt, B. M., Bryant, C. A., Mitton, D. R., Larsen, S. A., & Wilkinson, C. W. (2000). Chronic daily ethanol and withdrawal: 1. long-term changes in the hypothalamo-pituitary-adrenal axis, Alcoholism: Clinical and Experimental Research, 24(12), 1836-1849.

Reul, J. M., & de Kloet, E. R. (1985). Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. Endocrinology, 117, 2505-2511.

Reed, A. L., Happe, H. K., Petty, F., & Bylund, D. B. (2008). Juvenile rats in the forced-swim test model the human response to antidepressant treatment for pediatric depression. Psychopharmacology, 197, 433-441.

Romeo, R. D., Bellani, R., Karatsoreos, I. N., Chhua, N., Vernov, M., Conrad, C. D., et al. (2006). Stress history and pubertal development interact to shape hypothalamic-pituitary-adrenal axis plasticity. Endocrinology, 147, 1664-1674.

Romeo, R. D., Lee, S. J., Chhua, N., McPherson, C. R., & McEwen, B. S. (2004a). Testosterone cannot activate an adult-like stress response in prepubertal male rats. Neuroendocrinology, 79, 125-132.

Romeo, R. D., Lee, S. J., & McEwen, B. S. (2004b). Differential stress reactivity in intact and ovariectomized prepubertal and adult female rats. Neuroendocrinology, 80, 387-393.

Sarabdjitsingh, R. A., Meijer, O. C., Schaaf, M. J. M., & De Kloet, E. R. (2009). Subregion-specific differences in translocation patterns of mineralocorticoid and glucocorticoid receptors in rat hippocampus. Brain Research, 1249, 43-53.

Schmidt, M. V., Sterlemann, V., Ganea, K., Liebl, C., Alam, S., Harbich, D., et al. (2007). Persistent neuroendocrine and behavioral effects of a novel, etiologically relevant mouse paradigm for chronic social stress during adolescence. Psychoneuroendocrinology, 32(5), 417-429.

Seib, L. M. & Wellman, C. L. (2003). Daily injections alter spine density in rat medial prefrontal cortex. Neuroscience Letters, 337, 29-32.

Shepard, J. D., Barron, K. W., & Myers, D. A. (2000). Corticosterone delivery to the amygdala increases corticotropin-releasing factor mRNA in the central amygdaloid nucleus and anxiety-like behavior. Brain Research, 861, 288-295.

Shaffery, J. P., Lopez, J., Bissette, G., & Roffwarg, H. P. (2006). Rapid eye movement sleep deprivation in post-critical period, adolescent rats alters the balance between inhibitory and excitatory mechanisms in visual cortex. Neuroscience Letters, 438, 131-132.

Sibille, E., Sarnyai, Z., Benjamin, D., Gal, J., Baker, H., & Toth, M. (1997). Antisense inhibition of 5-hydroxytyptamin2a receptor induces an antidepressant-like effect in mice. Molecular Pharmacology, 53(6), 1056-1063.

Silveri, M. M. & Spear, L. P. (2004). Characterizing the ontogeny of ethanol-assiciated increases in corticosterone. Alchohol, 32, 145-155.

Sousa, N., Madeira, M. D., & Paula-Barbosa, M. M. (1998). Effects of corticosterone treatment and rehabilitation on the hippocampal formation of neonatal and adult rats. An unbiased stereological study. Brain Research, 794, 199-210.

Spear, L. P. (2000a). Neurobehavioral changes in adolescence. Current Directions Psychological Science, 9, 111-114.

Spear, L. P. (2000b). The adolescent brain and age-related behavioral manifestations. Neurosci Biobehavioral Reviews, 24, 417-463.

Spencer, R. L., Young, E. A., Coo, P. H., & McEwen, B. S. (1990). Adrenal steroid type I and type II receptor binding: estimates of in vivo receptor number, occupancy, and activation with varying level of steroid. Brain Research, 514, 37-48.

Spiga, F. & Lightman, S. L. (2009). Dose-dependent effects of corticosterone on nuclear glucocorticoid receptors and their binding to DNA in the brain and pituitary of the rat. Brain Research, 1293, 101-107

Sterlemann, V., Ganea, K., Liebl, C., Harbich, D., Alam, S., Holsboer, F., et al. (2008). Long term behavioral and neuroendocrine alterations following chronic social stress in mice: Implications for stress-related disorders. Hormones & Behavior, 53(2), 386-394.

Sterner, E. Y. & Kalynchuk, L. E. (2010). Behavioral and neurobiological consequences of prolonged glucocorticoid exposure in rats: relevance to depression. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 34, 777-790.

Stone, E. A. & Quartermain, D. (1997). Greater behavioral effects of stress in immature as compared to mature male mice. Physiology & Behavior, 63(1), 143-145.

Tejani-Butt, S., Kluczynski, J., & Pare, W. P. (2003). Strain-dependent modification of behavior following antidepressant treatment. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 27, 7-14.

Toledo-Rodriguez, M., & Sandi, C. (2007). Stress before puberty exerts a sex- and age-related impact on auditory and contextual fear conditioning in the rat. Neural Plasticity, 2007, 71203.

Toth, E., Gersner, R., Wilf-Yarkoni, A., Raizel, H., Dar, D. E., Richter-Levin, G., et al. (2008). Age-dependent effects of chronic stress on brain plasticity and depressive behavior. J Neurochemistry, 107, 522-532.

Tsoory, M., Cohen, H., & Richter-Levin, G. (2007). Juvenile stress induces a predisposition to either anxiety or depressive-like symptoms following stress in adulthood. European Neuropsychopharmacology, 17, 245-256.

Tsoory, M., & Richter-Levin, G. (2005). Learning under stress in the adult rat is differentially affected by 'juvenile' or 'adolescent' stress. International Journal of Neuropsychopharmacology, 8, 1-16.

Uys, J. D. K., Marais, L., Faure, J., Prevoo, D., Swart, P., Mohammed, A. H., et al. (2006a). Developmental trauma is associated with behavioral hyperarousal, altered HPA axis activity, and decreased hippocampal neurotrophin expression in the adult rat. Annals of the New York Academy of the Sciences, 542-546.

Uys, J. D. K., Muller, C. J. F., Marais, L., Harvey, B. H., Stein, D. J., & Daniels, W. M. U. (2006b). Early life trauma decreases glucocorticoid receptors in rat dentate gyrus upon adult re-stress: Reversal by escitalopram. Neuroscience, 137, 619-625.

Vazquez, D. M. & Akil, H. (1993). Pituitary-adrenal response to ether vapor in the weanling animal: characterization of the inhibitory effect of glucocorticoids on adrenocorticotropin secretion. Pediatric Research, 34(5), 646-653.

Vyas, A., Mitra, R., Shankaranarayana Rao, B. S., & Chattarji, S. (2002). Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. Journal of Neuroscience, 22, 6810-6818.

Vyas, A., Pillai, A. G., & Chattarji, S. (2004) Recovery after chronic stress fails to reverse amygdaloid neuronal hypertrophy and enhanced anxiety-like behavior. Neuroscience, 128, 667-673.

Walker, C. D., Trottier, G., Rochford, J., & Lavallee, D. (1995). Dissociation between behavioral and hormonal responses to the forced swim stress in lactating rats. Journal of Neuroendocrinology, 7, 615-622.

Wall, P. M., & Messier, C. (2001). Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior. Neuroscience & Biobehavioral Reviews, 25, 275-286.

Watanabe, Y., Gould, E., Daniels, D. C., Cameron, H., & McEwen, B. S. (1992). Tianeptine attenuates stress-induced morphological changes in the hippocampus. European Journal of Pharmacology, 222, 157-162.

Watanabe, M., Tamano, H., Kikuchi, T., & Takeda, A. (2010). Susceptibility to stress in young rats after 2-week zinc deprivation. Neurochemistry International, 56(3), 410-416.

Wellman, C. L. (2001). Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after corticosterone administration. Journal of Neurobiology. 49(3), 245-253.

Woolley, C., Gould, E., & McEwen, B. S. (1990). Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. Brain Research, 531, 225-231.

Wright, L. D., Hebert, K. E., & Perrot-Sinal, T. S. (2008). Periadolescent stress exposure exerts long-term effects on adult stress responding and expression of prefrontal dopamine receptors in male and female rats. Psychoneuroendocrinology, 33, 130-142.

Zamudio, S. R., Quevedo-Corona, L., Garces, L., & Cruz, F. D. L. (2009). The effects of acute stress and acute corticosterone administration on the immobility response in rats. Brain Research Bulletin, 80, 331-336.

Appendix A: Supplemental Results

Experiment 1

EPM

In the MANOVA for time in the open arm, ratio of open arm to total arm entries, time in the center, closed arm entries, head dips, rears and latency to the open arm with treatment and time of testing as the between subjects factors for adolescents there was no effect of treatment on time in the open arm, $F(1,46)=0.03$, $p=0.86$, ratio of open arm to total arm entries, $F(1,46)=3.55$, $p=0.07$, time in the center, $F(1,46)=0.01$, $p=0.93$, closed arm entries, $F(1,46)=1.49$, $p=0.23$, head dips, 0.77, $p=0.39$, rears, $F(1,46)=0.78$, $p=0.38$, or latency to the open arm, $F(1,46)=0.05$, $p=0.83$.

A MANOVA for time in the open arm, ratio of open arm to total arm entries, time in the center, closed arm entries, head dips, rears and latency to the open arm with treatment and time of testing as the between subjects factors showed that adults had no effect of treatment on time in the open arm, $F(1,49)=0.94$, $p=0.34$, ratio of open arm to total arm entries, $F(1,49)=1.92$, $p=0.17$, time in the center, $F(1,49)= 0.03$, $p=0.87$, closed arm entries, $F(1,49)= 0.002$, $p=0.96$, head dips, $F(1,49)=3.64$, $p=0.06$, rears, $F(1,49)=1.30$, $p=0.26$, or latency to the open arm, $F(1,49)=1.19$, $p=0.28$. There was no effect of time of testing on time in the open arm, $F(1,49)=3.51$, $p=0.07$, ratio of open arm to total arm entries, $F(1,49)=0.29$, $p=0.59$, time in the center, $F(1,49)=2.54$, $p=0.12$, closed arm entries, $F(1,49)=0.93$, $p=0.34$, rears, $F(1,49)=0.28$, $p=0.60$ and latency to the open arm, $F(1,49)=0.48$, $p=0.49$.

Experiment 2

FST

Latency to immobility: For adolescents there was a significant effect of time of testing on latency to immobility, such that the rats tested for enduring effects took less time to become immobile than the adolescents tested immediately following treatment, $F(1,30)=22.94$, $p<0.000$, but not for adults, $F(1,30)=0.97$, $p=0.33$.

Climbing: For the adolescents there was a significant interaction between time of testing and time point in the test, $F(3,90)=7.43$, $p<0.000$, and the same was true for the adults $F(3,90)=4.74$, $p=0.004$. There was also a main effect of time point such that the rats climbed less and less throughout the test, $F(3,90)=197.96$, $p<0.000$.

Appendix B: Ethics

Brock University

Animal Care and Use Committee (ACUC)
Chair – Rene Vandenboom, PhD 905.688.5550 ext 4726
Clinical Veterinarian – Alistair Ker, D.V.M. 905.227.7644
Animal Care Technician – Dayle Belme, ACT, M.Sc. 905.688.5550 ext 5820/3140

Date: Dec 18/08

Dear Dr. McCormick and Ms. Waters,

Your "Animal Use Project Proposal (AUPP)" entitled: **Investigation of immediate and lasting effects of chronic elevation of glucocorticoid hormones in adolescence compared to adulthood on hypothalamic-pituitary-adrenal function and anxiety-like behaviour.**

has been approved by the Animal Care and Use Committee. This approval expires in one year on the last day of the month. The number for this project is **AUPP # 08 - 11 - 02** .
This number must be indicated when ordering animals for this project.

ANIMALS APPROVED: **96 Long Evans rats**

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures, as described in this protocol, are familiar with the contents of this document.

Rene Vandenboom, Chair of ACUC

THIS PROTOCOL IS IN EFFECT FOR A PERIOD OF ONE YEAR ONLY.

Brock University

Animal Care and Use Committee (ACUC)
Chair –Rene Vandenboom, PhD 905.688.5550 ext 4726
Clinical Veterinarian – Alistair Ker, D.V.M. 905.227.7644
Animal Care Technician – Dayle Belme, ACT, M.Sc. 905.688.5550 ext 5820/3140

Date: Jan 28/10

Dear Dr. McCormick and Ms. Waters,

Your "Animal Use Project Proposal (AUPP)" entitled:

INVESTIGATION OF IMMEDIATE AND LASTING EFFECTS OF CHRONIC INJECTION IN ADOLESCENCE COMPARED TO ADULTHOOD ON HYPOTHALAMIC-PITUITARY-ADRENAL FUNCTION AND DEPRESSION-LIKE BEHAVIOUR.

has been approved by the Animal Care and Use Committee. This approval expires in one year on the last day of the month. The number for this project is **AUPP # 09 - 12 - 03**.
This number must be indicated when ordering animals for this project.

ANIMALS APPROVED 63 long evans rats

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures, as described in this protocol, are familiar with the contents of this document.

Rene Vandenboom, Chair of ACUC

THIS PROTOCOL IS IN EFFECT FOR A PERIOD OF ONE YEAR ONLY.